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Distribution of potato cyst nematodes in England and Wales and the use of 1,3-dichloropropene for their control

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A thesis submitted in partial fulfilment of the requirements of the Open University for the degree of Doctor of Philosophy

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Distribution of potato cyst nematodes in England and Wales and the use of 1,3-dichloropropene for their control

Abstract

In the UK the most problematic pests of the potato crop are the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. A structured survey of potato growing land in England and Wales was undertaken to reassess their occurrence and distribution. PCN were present in 64% of sites sampled and of the populations found, 67% were *G. pallida*, 8% were *G. rostochiensis* and 25% contained both species. Just over 50% of the sites sampled had a rotation length of 1 in 5 or less and cultivars with partial resistance to *G. pallida* represented only 6% of the total number of plantings while those with resistance to *G. rostochiensis* represented 43%. The results show an increase in the incidence of PCN compared with previous surveys and confirm the perceived shift towards *G. pallida* as the predominant species.

Two field experiments were done to look at the integration of methods for the control of PCN and subsequent reduction in yield loss in situations of very high PCN levels. The first experiment assessed the use of 1,3-dichloropropene (1,3-D) with the granular nematicides aldicarb, oxamyl and fosthiazate when growing the susceptible cultivar Estima. The second experiment assessed the use of the resistant cultivar Santé with 1,3-D and oxamyl at full and half-rates. 1,3-D significantly advanced emergence, increased percentage ground cover, root invasion, yield, tuber numbers, and improved tuber size distribution. Nematode multiplication was significantly reduced by fumigation in the first experiment.

The use of 1,3-D for the control of weed seeds was assessed and the results show a reduction in germination of weed seeds in soil after fumigation. The use of 1,3-D for the control of *Rhizoctonia solani* was also assessed and a trend in reduction in *R. solani* was observed after fumigation although the differences were not statistically significant.

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Statement of advanced studies

During the tenure of this project, in addition to performing and reporting the experiments in this manuscript the author has also:

- received training for cyst identification and species determination by morphological methods
- completed an MSc course in statistical methods
- attended weekly research seminars at Harper Adams University College and given a presentation
- gave presentations at the postgraduate colloquiums at Harper Adams University College in 1997, 1998 and 1999.
- attended The Association of Applied Biologists- Offered Papers in Nematology, Linnean Society, London, 17 December 1997
- attended the 24th International Nematology Symposium of the European Society of Nematologists, Dundee, Scotland, 4-9 August 1998
- presented a poster at The Association of Applied Biologists- Offered Papers in Nematology, Linnean Society, London, 17 December 1998
- presented a paper at The Association of Applied Biologists- Offered Papers in Nematology, Linnean Society, London, 14 December 1999
- presented a paper at 25th International Symposium on Nematology of the European Society of Nematologists, Herzliya, Israel, 2-7 April 2000
- presented a paper at The Association of Applied Biologists- Potato Cyst Nematode Management, Harper Adams University College, 6 June 2000
- gave demonstrations to BSc students on cyst extraction and quantification
- gave a presentation explaining Field Experiment Two at Harper Adams Open Day

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1.0 Chapter 1.

Introduction

1.1 Origin and dissemination of potato cyst nematodes

Potatoes have been cultivated in the high Andes region of South America for thousands of years. It is thought that potato cyst nematodes (PCN) evolved with the potato plant and other tuberous species of *Solanum* at altitudes of approximately 2 000 m or more (Jones & Parrott, 1968; Ellenby & Smith, 1968). Potatoes were introduced into Europe about 1570 (Evans, Franco & De Scurrah, 1975) but it was not until the 1880s that PCN were first noticed by Kühn in Germany (Kühn, 1881). The precise pathway of introduction of PCN from South America to Europe must remain a matter of speculation (Franco, Oros, Main & Ortuno, 1998). However, various historical-geographical events that occurred during and after the discovery of the New World may have been instrumental in the introduction.

In the 1840s, outbreaks of potato blight (*Phytophthora infestans*) caused widespread famine, notably in Ireland. Many tubers were subsequently imported from South America into Europe, to be used in breeding programmes to incorporate blight resistance, and it is thought that PCN may have been introduced on tubers brought from South America around 1850 (Jones, 1970).

Inagaki & Kegasawa (1973) found viable cysts of *G. rostochiensis* in Peruvian guano fertiliser that had been imported to Japan in 1969 from islands located off the coast of Peru. They suggested that importation of this fertiliser could have been a means by which PCN reached England and Germany. They suggested that birds carried the nematodes from the high Andes to the offshore islands but this has since been deemed unlikely (Franco *et al.*, 1998). However, it is likely that guano was shipped to England and Germany in old PCN-contaminated potato bags, so PCN may have been introduced in the 1840s onwards (Franco *et al.*, 1998) which would agree with when they were first noticed in Europe.

There are six recognised species of *Globodera* which are parasites of the Solanaceae (Evans & Stone, 1977; Evans & Rowe, 1998). Two of these species are pests of potatoes in the UK: *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 and *G. pallida* Stone, 1973.

A study of PCN distribution in South America carried out by Evans *et al.* (1975) found that the two species were present in different geographical areas and latitudes: *G. rostochiensis* was found at more southerly latitudes in Bolivia, whereas *G. pallida* was found in Columbia, Ecuador and most of Peru. In some areas of Peru, however, the two species were found together. These findings probably broadly reflect the original distribution of the two species. Further work by Evans & Franco (1977) confirmed the northern limit for *G. rostochiensis* as 15.6 degrees south. It is thought that only a restricted part of the South American gene pool has been introduced to many countries (Evans & Trudgill, 1992).

1.1.1 Biology of PCN

The two species of potato cyst nematode are closely related and were only separated by Stone (1972), with *G. pallida* gaining its designation due to the pale colour of its developing cysts as compared to *G. rostochiensis,* which has golden coloured cysts. Prior to this, both species were referred to as *Heterodera rostochiensis* (Evans & Franco, 1977; Schots, Bakker, Gommers & Egberts, 1988). The two species have similar biologies (although they do differ in certain important characteristics) and cause similar damage and symptoms (Trudgill, Phillips & Alphey, 1987).

PCN are highly specialised sedentary endoparasites. When mature, the females are spherical whereas the males are vermiform. Once a host crop is harvested, the fertilised females die and their bodies undergo a tanning process to become hardened cysts, which contain up to 400 eggs (Brodie, Evans & Franco, 1993).

The majority of the dormant juveniles in the cyst only hatch when stimulated by specific exudates (hatching factors) released by a host crop. In the presence of a host 60-80% of the eggs hatch (Rawsthorne & Brodie, 1986), while in the years when non-host crops are grown about 25-30% of the eggs will generally either hatch or die within the cyst (Jones, 1970).

When the juveniles hatch, they invade the roots of a host plant close to the root tip. They cut their way through the cortical cells until they reach the stele, where they cause specialised transfer cells (syncytia) to be formed (Jones & Northcote, 1977). The syncytia are formed following the breakdown of the cell walls of adjacent phloem parenchyma cells. The juveniles become sedentary and feed on the syncytia and develop into adult males or females. The males, unlike the females, do not remain sedentary and, when mature, leave the roots and enter the soil. They are attracted by pheromones to females, which they fertilise. Once the female is fertilised, she forms eggs within her own body, dies and forms a new cyst. The mature cyst and the adult female are spherical, about 0.5 mm in diameter, and are the only stages in the life-cycle visible with the naked eye.

1.1.2 Pest status

Several factors contribute to the pest status of PCN. Cysts can remain viable in the soil for up to 25 years (Grainger, 1951; Evans & Trudgill, 1992; Turner, 1996*a*). The eggs

contained within the cysts can withstand drying, unlike the eggs of most other nematode species (Evans & Trudgill, 1992).

Another major factor is that PCN are capable of a population increase of up to seventy-fold in one season (Evans & Trudgill, 1992). Combined with their slow rate of decline in the absence of a host, when only 20-30% of eggs hatch or die per year, this makes them very persistent. Being soil-borne, PCN are extremely difficult to control since all chemicals require thorough incorporation into the soil to come in contact with all of the nematodes. And, of course, the over-riding determinant of PCN as pests lies in the damage caused to the crop, which can be severe.

1.1.3 The pathotype scheme

Following the discovery of resistance in potatoes to PCN, it was found that some PCN populations were able to multiply on potatoes which had resistance to other populations. As a response to these observed differences, systems were devised to classify these populations into different "pathotypes". There were separate schemes operating in The Netherlands, the Federal Republic of Germany and the United Kingdom. The most recent pathotype scheme was introduced by Kort, Ross, Rumpenhorst & Stone (1977) and was proposed as an international scheme. An alternative proposal from South America was made by Canto Saenz & De Scurrah (1977) but their scheme is not generally used in Europe.

The pathotypes were defined as variants of a PCN species, which differ from others by their ability to multiply on particular potato genotypes used as differential hosts. Pathotypes within *G. rostochiensis* were prefixed with Ro and those of *G. pallida* with Pa.

Under the Kort *et al.* (1977) scheme, there were five pathotypes of *G. rostochiensis* numbered Ro1 to Ro5 and three of *G. pallida* numbered Pa1 to Pa3. In the South American scheme of Canto Saenz & De Scurrah (1977), five pathotypes of *G. pallida* are recognised, with P4A and P5A being equivalent to the European Pa2 and Pa3.

The Kort *et al.* (1977) scheme was critically reviewed by Trudgill (1985), who proposed that many of the pathotypes were in fact artefacts. The scheme was flawed since many of the populations were classified according to their multiplication rates on partially resistant differential genotypes and the results could vary greatly due to environmental effects. He suggested that most European populations are mixtures of pathotypes and heterogeneous for virulence genes and that a new scheme was needed. Trudgill (1985) suggested that the best definition of a pathotype to use was that of Andersen & Andersen (1982). They proposed three criteria for a pathotype:

1) the genetic background for virulence in the nematode and resistance in the plant is known
2) there are significant differences in virulence between pathotypes
3) all members of a pathotype should have one gene, or group of genes, for virulence that is common to all individuals of the pathotype and is different from the genes or gene combinations found in any other pathotype.

Some of the pathotypes that Kort *et al.* (1977) proposed are valid if the definition of Andersen & Andersen (1982) is used. The pathotypes Ro1 and Ro4 of *G. rostochiensis* together and Pa1 of *G. pallida* satisfy the criteria set out and are homozygous for the avirulence genes to the H_1 and H_2 resistance genes respectively. Pathotypes Ro2, Ro3 and

Ro5 are heterogeneous mixtures of virulence types, as are Pa2 and Pa3 (Phillips *et al.*, 1992).

Nijboer & Parlevliet (1990) concluded that there are three recognisable pathotypes of *G. rostochiensis* and suggested that they be renamed Ro1 (old Ro1 and Ro4), Ro3 (old Ro2 and Ro3) and Ro5 (old Ro5). It seemed impossible to reliably identify pathotypes of *G. pallida.*

In the UK, all the individuals and populations of *G. rostochiensis* lack the recessive gene conferring virulence against the H_1 gene for resistance and are classified as Ro1 (Brodie, *et al.*, 1993). Thus there is a single gene for resistance in the plant and for virulence in the nematode (Mugniery *et al.*, 1989). However, Trudgill (1985) concluded that most European populations of PCN are heterogeneous for virulence genes.

1.1.4 Crop damage

The damage caused by both species is similar and is usually first noticed in patches around the focus of the original introduction. The symptoms of attack are similar to those caused by water logging, acidity, poor soil and lack of nutrients (Jones & Jones, 1984), and manifested as stunting, chlorosis and wilting. The mechanisms of damage have been extensively studied (Evans & Trudgill, 1992). Much of the damage is caused by the invading juveniles which destroy root cells and inhibit root growth (Evans 1982; Storey 1982). The main roots are shorter but may have more lateral branches, which penetrate into a smaller volume of soil than normal. Root damage makes the plants less efficient at water and nutrient uptake (Brodie *et al.*, 1993). Consequently, affected plants have reduced top growth and are more prone to wilting. The foliage becomes pale and yellow and the crop is

more susceptible to competition from weeds. This is especially true if there is stress from high temperatures, bright sunlight or drought. Damaged plants have fewer and shorter stems but usually the same number of leaves per stem. The decrease in top fresh weight is proportionally greater than that of the root system (Evans, 1982). The reduction in top growth decreases the amount of solar radiation intercepted by the leaves. With high PCN populations, there will be severe stunting of the crop with likely premature death and the possibility of near-total yield loss (Brodie *et al.*, 1993). By the time the symptoms of attack appear, considerable damage will have been done. The maturing cysts are easily identified in the field on the roots of infected plants.

The yield loss in potatoes susceptible to PCN is related to the initial population density. Brown (1969) found that, in twenty one trials on organic soils, tuber loss varied between 0.4 and 4.0 t ha^{-1} per 20 eggs g^{-1} soil. Brown & Sykes (1983) found that the losses attributed to PCN were even more varied than those previously reported and exceeded 20 t ha^{-1} at a level of 160 eggs g^{-1} soil. This work was done before the two PCN species had been recognised by Stone (1972) and it is therefore unknown which species, or a mixture of both species, was present on the sites used. Brown & Sykes (1983) reported that most of this earlier work was probably done on sites infested with *G. rostochiensis*. Brown (1969) showed that yield loss was dependent on the density of the initial nematode population before planting with the population densities on the sites sampled ranging from 0-456 eggs g^{-1} soil. Evans (1993) found that when PCN are few, yield is little affected because plants compensate for the trivial injury to their root systems, but as their numbers increase, yield declines and reaches a minimum. The value of the minimum yield depends largely on an interaction between the cultivar grown and soil type (Evans, 1993). The experiments done by Brown (1969) and Brown & Sykes (1983) used eight cultivars and it is now known that

the tolerance of a cultivar to nematode attack has a major influence on yield (Evans & Haydock, 1990). Therefore, differences in the initial population densities, soil type and cultivar would all contribute to the differences in the yield losses caused by PCN. Estimates of the maximum potential yield on maincrop potatoes grown in Europe range from 90 t ha^{-1} (Alcock, 1967) through 100 t ha^{-1} (van der Zaag & Burton, 1978) to even 115 t ha^{-1} (Allen & Scott, 1980). Yields of 90 t ha^{-1} were achieved by Gunn (1978). Such yields are uneconomic due to the law of diminishing returns since maximum profit is achieved at lower input levels and lower yields (Evans & Haydock, 1990).

1.2 Diagnostics

The conservative morphology of nematodes makes identification of many pest species demanding. Traditional diagnostic tests based on morphology are time consuming and expensive (Fleming & Powers, 1998). As a result, diagnostic tests based on differences in proteins, lipids, carbohydrates and deoxyribonucleic acid (DNA) have been developed.

1.2.1 Viability tests

A problem of monitoring PCN is deciding when eggs and juveniles are dead. This is particularly important for statutory and quarantine purposes. Various methods have been tried but none is entirely satisfactory (Shepherd, 1986). One method of differentiating between living and dead nematodes is by staining. Appropriate vital stains include New Blue R (Shepherd, 1962) and Meldola Blue (Ogiga & Estey, 1975). However, these stains only give reliable results when the semipermeability of the nematode's internal membranes has been broken down. This may not occur until several weeks after nematicide treatment (Shepherd, 1986). Other methods of viability testing include cutting each nematode to see if the body contents are expelled (Fielding, 1951) or applying electric currents across the

nematode (Granek, 1976). These methods would only be feasible for a few individuals. An ELISA test which combined species differentiation and viability testing would be efficient, time saving and give immediate answers.

1.2.2 Differentiation between species

It is possible to use isoelectric focusing on polyacrylamide gels to separate proteins from the two species of PCN and thereby differentiate between species, even using single cysts (Fleming & Marks, 1983; Fox & Atkinson, 1984). The proteins are separated in a pH gradient, with each protein moving through the pH gradient until it reaches the point at which it has reached its isoelectric point (pI). The protein then stops moving and each component of a protein mixture is concentrated at its pI position. Two bands have proved particularly useful in species identification. One band at pI 5.9 is found only in *G. rostochiensis* and another of pI 5.7 is found only in *G. pallida* (Marks & Fleming, 1985).

1.2.3 Differentiation between pathotypes

Many attempts have been made using biochemical techniques to find markers to differentiate between pathotypes. However, with the possible exception of certain *G. rostochiensis* pathotypes, there is no biochemical means of differentiating pathotypes. If the pathotype scheme is flawed, with no clear distinction between pathotypes, the absence of specific markers is not surprising. In an attempt to improve the scheme it was suggested that the original introductions might form a basis for classification and effort has concentrated on trying to find natural groupings (Phillips *et al.*, 1992). Molecular techniques are more sensitive in revealing intra-species variation and have been used to look for relationships between PCN populations. DNA restriction fragment length polymorphisms (RFLPs) have been used by Schnick, Rumpenhorst & Burgermeister

(1990) and Phillips *et al.* (1992). Schnick *et al.* (1990) were able to differentiate between 21 populations of *G. pallida* but markers that group the populations according to their virulence characteristics or pathotype designation have not been produced with this technique.

1.2.4 Differentiation between species using monoclonal antibodies

Schots *et al.* (1988) were able to differentiate between *G. rostochiensis* and *G. pallida* by producing monoclonal antibodies which reacted with thermostable proteins from both species. A number of different types of diagnostic tests use monoclonal antibodies (MAbs), the most widely used being the enzyme-linked immunosorbent assay (ELISA). A MAb binds to a target molecule unique to the organism and an enzyme-induced colour change confirms the presence of the target organism in the sample (Curran & Robinson, 1993). The intensity of the colour is proportional to the amount of antigen present and is measured spectrophotometrically.

For a quantitative ELISA, the choice of antigen for the MAb to recognise must reflect the population density of the nematode. In addition, the antigen has to be present at the appropriate time in the life-cycle (Curran & Robinson, 1993). Schots, Gommers & Egberts (1992) developed a quantitative ELISA for the detection of PCN in soil samples. However, the MAbs produced were not species-specific because they cross-reacted with the two species of PCN and other nematode species. The MAbs produced by Robinson *et al.* (1993) did not have this problem.

Robinson *et al.* (1993) described two MAbs that differentially recognise the two species of PCN. They recognise proteins of the same molecular weight (34kD) in both species. These

proteins have isoelectric points at pH values of 5.7 in *G. pallida* and 5.9 in *G. rostochiensis*, and are the same proteins as were used by Marks & Fleming (1985). The antibodies reacted strongly only with *Globodera* species and were therefore suitable to be used in a quantitative immunoassay for PCN. The immunoassay was further developed in work reported by Evans, Curtis, Robinson & Yeung (1995), when it was noted that non-viable components of the cyst such as the cyst wall and eggshell had no antigenicity and that dead eggs lost their antigenicity. This is important for a quantitative immunoassay which is designed to estimate the number of live PCN in a given amount of soil (Evans *et al.*, 1995). Davies, Curtis & Evans (1996) noted that the levels of cross-reactivity of the MAbs were sufficiently low to make them irrelevant. It was possible to identify samples containing mixed populations of PCN. Davies *et al.* (1996) commented that it was possible for an immunoassay to combine identification and quantification in a single step and to have the potential for automation.

1.2.5 Differentiation between species using the polymerase chain reaction

Several attempts have been made recently to distinguish between cyst nematode populations by using the polymerase chain (PCR) reaction (Mulholland *et al.*, 1996; Bulman & Marshall, 1997). PCR techniques offer the prospect of a simple, rapid and reliable diagnostic tool to enable the identification and quantification of PCN from field samples.

1.3 PCN in the UK

There are thought to have been relatively few introductions of PCN directly from South America into other countries. Both species have spread to many potato growing areas of the world but it is thought that Europe has acted as a secondary distribution centre (Evans

& Trudgill, 1992). They have been reported in 65 countries (EPPO, 1994) with *G. pallida* in 41 of these countries. No country seems to have received introductions of only *G. pallida.* Only part of the gene pool of PCN has been introduced into the UK. This has implications for the type of resistance required for control (Mugniery *et al.*, 1989) and for PCN management, since programmes for control and resistance breeding depend on the identification of species, pathotypes or populations (Schnick *et al.*, 1990).

1.3.1 Dispersal of PCN

Dispersal occurs when PCN are in the cyst stage of their life cycle (Evans & Trudgill, 1992). Cysts can be spread over long distances in soil or by the agencies of wind or water, including drainage or flooding (Evans & Trudgill, 1992). Brodie (1993) noted that spread of an infestation between fields on a local level was due to normal farming activity. Spread over long distances was possible in soil carried on machinery, seed tubers or other root crops. Cysts can be spread on the surfaces of tubers or in soil in seed bags or clinging to tubers. They can also be spread by transplants of other plants with soil on their roots, for example brassica transplants (Evans & Trudgill, 1992). Inagaki & Kegasawa (1973) concluded that Peruvian guano fertiliser, imported to Japan in 1969 was a likely means by which PCN had been introduced to Japan.

Dispersal on soil adhering to farm machinery is hard to control even with a strict cleaning regime. Evans & Brodie (1980) mention the introduction of *G. rostochiensis* into the USA from Europe on military equipment brought back after the second world war. Brodie (1993) found that spread of *G. rostochiensis* was possible from infestations undetectable with current soil sampling techniques and Evans & Brodie (1980) suggested that most spread occurs from infestations that are not detectable by a soil survey. After dispersal, the

small number of nematodes spread will need a suitable host to multiply on before detection is possible.

1.3.2 History of potato cropping in the UK

The area of potatoes planted in Great Britain between 1865 and 1940 fluctuated narrowly between 200 000 and 280 000 ha but the tillage area decreased throughout this period, with the frequency of cropping increasing (Jones, 1970). After 1940, the hectorage and frequency of planting both increased to reach a peak of about 520 000 ha in 1948 (due to the demand for potatoes caused by the Second World War) such that potatoes were sometimes grown in a rotation of two crops in three years (Dixon *et al.*, 1968). However, the build up of high PCN populations in south west Lancashire and south Lincolnshire caused plantings to be reduced in these regions. Although the area planted decreased in south Lincolnshire, the number of fields found to be moderately or highly infested continued to increase up to 1955, and even after this, the number of slightly infested fields also increased. After the peak in 1948, the area of potatoes planted had declined to around 240 000 ha by 1970 and by 1999 had declined further to about 140 000 ha (British Potato Council, personal communication).

The number of farmers producing potatoes has also fallen steadily over the last forty years from almost 80 000 in 1960 to fewer than 10 000 by 1998 (Walker, 1998). Individual farmers have become more specialised and grow more potatoes on a smaller area of land partly due to shorter rotations. At the same time, yields per hectare have increased from an average of 25 t ha^{-1} in the 1960s up to about 45 t ha^{-1}. This rationalisation can be summed up by the statistic that 20% of farmers now grow 80% of the output (Walker, 1998).

1.3.3 History of PCN in the UK

Massee (1913) first reported cyst-nematodes on potatoes in Scotland. The cysts were thought to be *Heterodera schachtii* and it was reported that they proved destructive to potatoes with the rootlets attacked in a similar way to those of sugar beet being attacked by *Heterodera schachtii.* Since potatoes are now known not to be a host crop for *Heterodera schachtii,* this must have been the first reporting of a *Globodera* species in this country. Warburton (1919) noted in his Annual Report for 1919 of the Zoologist that "a few cases of infestation by the root-knot eelworm (*Heterodera schachtii*) have been reported".

Strachan & Taylor (1926) found that increasing attention was being paid to potato eelworm, which affected potato crops in many parts of the country. They also noted it was similar to "beet eelworm" and referred to it as "potato eelworm". In the East Riding of Yorkshire, the effects of potato eelworm had been known for some considerable time. A farmer first noticed a small patch in the centre of a field in 1904 that had been the site of a potato clamp the previous year. The field was frequently cropped with potatoes and, by 1917, was completely infested. In addition, over a period of 21 years, symptoms were noted on three of the five arable fields on the farm (Strachan & Taylor, 1926).

Potato cyst nematodes were first recognised in Lancashire in 1927 (Smith & Prentice, 1929) although a potato disease that was confined to patches was known of in 1922. By 1930, it had become a major problem and caused a reduction in potato growth on individual farms. Dixon *et al.* (1968) commented that, at the end of the 19th century, the potato crop occupied a quarter of the arable area on the mineral and moss soils in south west Lancashire. At this time, and in the first half of the 20th century, PCN levels were

building up. It was reported increasingly in all potato growing areas from 1920 onwards (Jones, 1970).

Potato cyst nematodes were first recorded in Lincolnshire in 1924 (Morgan, 1925) but were probably known about before this time. When they first appeared in south Lincolnshire, some of the infested fields had grown potato crops for 20 years in succession (Jones, 1970). A survey carried out by Carslaw & Graves (1939) in Lincolnshire found that only 9 out of 45 growers visited had seen any evidence of PCN on their farms. Wood (1946) estimated that 20 per cent of the arable area of the county was infested. However, a survey done by Winfield (1965) of farms in Lincolnshire suggested that infestations were more widespread. A random selection of 100 growers was made and a soil sample was taken from one field per farm that was to be used to grow potatoes in the following year. The results showed that cysts were found in 84% of the fields sampled. This was comparable to tests done on routine advisory soil-samples collected during the period 1949-59, in which cysts were present in 84% of the fields sampled.

1.3.4 Distribution of PCN in the UK

Work has been done on the occurrence and distribution of PCN since they were first recognised as pests of potatoes. However, early studies were made before it was realised that there were two species. Jones & Pawelska (1963) tested forty seven PCN populations for their ability to form cysts on resistant potato clones using Arran Banner as a susceptible standard. Although it was to be another nine years before PCN were separated into the two species, the ability to multiply on a resistant cultivar differentiated between *G. rostochiensis,* which was unable to multiply, and *G. pallida* which was able to. They found that *G. rostochiensis* was especially common in East Anglia, south-east England and

Northern Ireland, with *G. pallida* more common in Yorkshire, the East Midlands and the Channel Islands. In all other regions, there was a mixture of populations but with more being "resistance-breaking". However, some of the *Solanum tuberosum* ssp. *andigena* hybrids suppressed multiplication of *G. pallida*, making the results less useful in terms of indicating the distribution of the species.

Guile (1967) conducted experiments and surveys to determine differences between populations and pathotypes and to assess their distribution in the East Midlands. He mapped pathotype A (now known as *G. rostochiensis)* and pathotypes B and C (now known as *G. pallida).* A crop survey showed that 82% of fields contained only pathotypes that had white and creamy-white females (*G. pallida*), which were most common in North Kesteven, Lindsey (Lincolnshire) and North Nottinghamshire. Pathotype A (with golden females) predominated in 7% of the fields, located mainly in the fens of South Kesteven. The remaining 11% were of mixed pathotypes. Guile concluded that pathotypes B and C (*G. pallida*) predominated in the East Midlands.

Dixon *et al.* (1968) conducted a local survey of pathotypes in south west Lancashire. From 37 populations tested for their resistance to *Solanum tuberosum* ssp. *andigena*, 11 of the populations were pathotype A (*G. rostochiensis*) and 26 were able to overcome the resistance (*G. pallida*).

Brown (1970) also surveyed populations by testing them on resistant potato cultivars derived from *Solanum tuberosum* ssp. *andigena.* He divided England and Wales into areas according to their ability to produce cysts on resistant compared to non-resistant cultivars. Non-resistance-breaking populations (*G. rostochiensis*) were found to be more

predominant on the peat soils of East Anglian fens and in Bedfordshire, Essex and Kent. Populations now known to be *G. pallida* were more common in the East Midlands and Yorkshire. Populations in the rest of England and Wales were found to vary in their ability to produce cysts on resistant cultivars.

Stone, Holliday, Mathias & Parrott (1986) found that all the *G. rostochiensis* populations tested in the UK were pathotype Ro1, and that a minority of *G. pallida* populations were pathotype Pa1 and many were Pa3. However, the differentiation between Pa2 and Pa3 was considered unsatisfactory.

1.3.5 Recent PCN surveys

There has been no systematic survey of the incidence of PCN in England and Wales for at least ten years (Hancock, 1996). Data from soil samples taken for statutory or advisory purposes have not been collated. Of the soil samples examined for PCN by ADAS for any reason from 1993 to 1995, about 36% contained viable PCN. These samples included those being tested to obtain a certificate of freedom from PCN for export of potatoes. Such samples would come from land thought to be free from PCN and the real level of infestation may be higher. If these statutory samples are excluded, a sub-set of soil samples sent to ADAS for advisory reasons in 1994 and 1995 suggests that about 67% of land cropped with potatoes is infested with PCN. In this analysis, about 27% of crops would give an economic yield response to nematicide (Hancock, 1996). This is similar to other data from samples collected from 1982-86 by ADAS at their Leeds, Shardlow, Kirton and Cambridge laboratories, in which 62% of samples contained PCN and 22% of crops would give an economic yield increase to nematicide (Hancock, 1988)

The Potato Marketing Board (PMB) made a subjective survey of PCN distribution in England, Wales and Scotland in 1992. The total area cropped with potatoes was 153 786 ha. Of this cropped land it was estimated that 42.1% was infested with PCN, i.e. about 65 000 ha involving approximately 40% of registered producers (Storey, personal communication). The estimated number of potato producers in 1992 was 16 539 and some 39.6% had land infested with PCN. The PMB found that the highest proportion of infested land and of growers with infested land were on the eastern side of England. The counties most affected were Bedfordshire, Cambridgeshire, Northamptonshire and Norfolk. About 78% of growers in this sub-set who sent in soil samples had viable cysts in their sample (Hancock, 1996).

The species of PCN present were determined for the ADAS samples sent in from 1993 to 1995 (Hancock, 1996). The results indicate that only 5% of fields contain populations that are predominantly *G. rostochiensis*. About 41% of samples contained both species and 54% contained predominantly *G. pallida*. The distribution of PCN in the 1960s by Brown (1970) showed that about half of the populations in England were pure *G. rostochiensis* (Evans, 1993). The repeated growing of resistant cultivars has reduced the occurrence of *G. rostochiensis* whilst allowing selective reproduction of *G. pallida*.

Hancock (1996) noted that although the predominant species in England and Wales was *G. pallida*, in East Anglia the dominant species was *G. rostochiensis*. Hancock (1996) concludes that we do not know the current distribution of PCN in England and Wales. The current estimate of around 40% of cropped land being infested with PCN is based on biased, unrepresentative data. A systematic survey would give better information on the occurrence and distribution of the two species.

1.4 Sampling for PCN

The objectives of nematode sampling include: general detection, surveys, diagnosis of disease problems, providing advice in integrated pest management programmes and research needs (Barker & Campbell, 1981). The objectives of sampling should be defined before a survey is started to give the best balance between reliability and efficiency. The accuracy of the estimate of PCN levels depends on the way that the soil is sampled and on the laboratory processing procedure.

Non-migratory cyst nematodes are usually distributed in arable land in a random pattern throughout the plough layer by cultivation (Boag & Neilson, 1994). Different techniques have been used to describe the amount of aggregation with the most common being the negative binomial distribution (McSorley, 1982; Seinhorst, 1982; Schomaker & Been, 1992). In the negative binomial distribution, the value k, which is a measure of aggregation, is dependent on the mean and to avoid this others have used Taylor's Power Law. This law can produce specific transformations for individual species (Boag & Topham, 1984).

Ward & Hockland (1996) discussed legislation and sampling strategies in decision making for nematode management. The Plant Health Service of MAFF co-operates with the European and Mediterranean Plant Protection Organisation (EPPO). *Globodera pallida* and *G. rostochiensis* are listed quarantine nematodes for the European Union and member states have made progress in harmonising their approach to their control.

There are two methods of sampling for the presence of PCN. The first involves taking soil samples from the field and processing them in the laboratory. The second involves lifting potato plants and looking for females or cysts (Anon, 1991).

1.4.1 Soil sampling

Since cysts are aggregated and not randomly distributed throughout a field, it is recommended that a soil sample is made up from sub-samples taken over the whole field. The patchy distribution of cysts results from the way that initial introductions are made. The initial foci are spread by cultivation to form secondary foci and eventually the whole field becomes infested. By the time an infestation is detectable, it is usually widespread over the field. The cropping pattern of a field may need to be taken into account when sampling as parts of a field may have been used more often for potatoes than others, thus increasing their chances of having a nematode infestation (Southey, 1974).

The first quantitative study on sampling for nematodes was made by Anscombe (1950). A subcommittee of the Conference of Advisory Entomologists discussed various techniques and recommended the following: 3.8 cm auger or a half cylindrical sampler of 2.5 cm diameter sampling to a depth of 20.3 cm. Fifty samples were recommended for fields up to 4 ha with the samples taken at random over the whole sampling area. Anscombe stated that the sampling procedure was unsatisfactory for inspection of individual seed-growing crops, but may prove useful for a survey of a seed district. The populations detectable by soil sampling were 0.01 cysts g^{-1} soil, which is equivalent to 24.7 million cysts ha^{-1}. He concluded that, with this procedure the presence of a serious infestation could be demonstrated but not its absence. Better confidence limits from soil sampling would require a great increase in effort.

Haydock & Evans (1994) discussed the use of soil sampling for decision making in PCN management. There are three basic requirements for the estimation of PCN levels in soil:

1) the soil sample must be large enough to achieve the required accuracy
2) the sample must be made up from enough sub-samples to be representative of the whole area sampled
3) the laboratory analysis must be efficient and as free from operator error as possible

1.4.2 Soil sample collection strategies

Turner (1993) investigated several soil sampling procedures for the detection of PCN. The processes examined included sampling equipment, sampling intensity and patterns of collection in order to make the detection as accurate and efficient as possible. Turner concluded that no sampling method is 100% efficient at detecting PCN in agricultural land. There will always be a compromise between an acceptable detection level and the resources available to collect and analyse the samples. Sample collection time is dictated primarily by how far the operator has to walk and not by the number of points sampled. There are a number of different ways of walking through the field. A sample collected from around the perimeter takes less time than a zigzag pattern. A cross-diagonal pattern and M-shaped pattern are also quicker. Turner concludes that, by the time an infestation has become detectable by normal sampling, it has already become distributed throughout the field. Therefore, taking numerous soil cores from many different points may be unnecessary. It was thought at first that a simple 'w' was sufficient but this left large parts of the field unsampled. Barker & Campbell (1981) recommended a zigzag pattern while Schomaker & Been (1992) recommended a grid.

Church, Gough & Southey (1959) carried out a study of systematic sampling on a grid pattern rather than random sampling. They found that sampling systematically at points on a regular grid could significantly reduce sampling errors. They found that accuracy increases with the number of sample points but, after a certain point, the increased effort becomes prohibitive. They advised: that samples should be made up from 50 points for an area up to 4 ha, sample size collected to be between 1 kg and 2.5 kg, and that 200 g of soil should be analysed from each bulk sample.

When larger soil samples are collected, it is common practice to process only a part of the sample after mixing. The errors due to sub-sampling of soil samples containing PCN were investigated by Been & Schomaker (1998). Several fields, infested with PCN were sampled by collecting bulk samples of approximately 70 cores giving 1.8 or 2.5 kg soil and sub-samples were processed separately to compare elutriation precision and accuracy and to check the quality of the mixing. They found that cysts appeared to be randomly distributed in the well mixed samples.

1.4.3 Auger design

Turner (1993) compared three augers by asking a number of different operators to sample the same field with each. Results confirmed that there was no difference in detection deficiency between the augers and it was concluded that 50 probes detected PCN as efficiently as 120 smaller probes.

1.4.4 Size of sample

The probability that a soil sample taken from an infested area will contain cysts depends on the population density of the cysts in the area being sampled, the amount of soil examined and on the number of points from which the soil was taken.

Southey (1970) stated that 50 cores taken in a half-cylindrical corer with blade 200 mm by 20-25 mm gave about 2 kg of soil. Southey (1974) discussed soil sampling as a method for detection and estimation of PCN and pointed out that small isolated infested patches that appear in a field are deceptive. In reality, by the time an infestation is detectable, it has been spread over the whole field but will still be undetectable in some places. The distribution within a field is dependent on previous cropping patterns that should be taken into account when sampling.

The EPPO quarantine procedure No. 30 (Anon, 1991) describes the recommended sampling strategy for PCN. Land that is to be used to grow seed potatoes must first be inspected by taking soil samples and declared free from viable PCN cysts. The sampling must be done after harvest of the previous potato crop. The EPPO recommendation for soil sampling is that a minimum of 50 cores are taken over an area of four ha or less, using a sampling tool which takes standard vertical borings in the cultivated top-soil. For statutory purposes, the amount of soil examined in the UK is 500 g. The standard corer used was a 'cheese-sampler' with blade 200 mm long and 20-25 mm diameter (Southey, 1974). A tube of 25 mm diameter and 200 mm long will remove almost 100 cm^3 but a half cylinder will remove less (McSorley, 1987).

1.4.5 Intensive soil sampling

Been & Schomaker (1996) developed a new method for detecting low populations of PCN to reduce nematicide use. The method detects small infestations with a predefined probability so eliminating the need for precautionary soil fumigation. Once a focus is found, this area alone is treated, thereby reducing pesticide application over the whole field. They found a more effective sampling method was based on a general model describing the size and shape of an infestation. The fields were first pre-sampled by dividing them into 8 by 3 m grids. The 8 m length was in the direction of cultivation. After analysing samples from within each of these boxes, any focus detected was more intensively sampled. This involved sampling every square metre and processing 1.5 kg soil to count cysts. They found all foci elliptical with population densities increasing exponentially towards the centre, but more slowly in the direction of cultivation than perpendicular to it. From this data, a model was developed which was found to have similar parameters in all growing areas and could be used to investigate the accuracy of a sampling method. A pilot version of the sampling method was introduced and evaluated and the total reduction in fumigant use in the Netherlands was 75% in 1994.

1.4.6 Rapid soil sampling

Cooke, McKinney & Thomason (1979) reported a rapid method for sampling surface soil using a motorised all-terrain cycle. The soil sampling unit was mounted at the rear and had cylindrical buckets bolted to a chain around three sprockets. A land wheel drives the top sprockets and the buckets collect surface soil as they pass beneath the bottom sprocket. Samples are collected every 2.3 m without the need to stop the cycle. A sample of 2.05 kg was collected from 300 points. It was found that the system did not operate satisfactorily on consolidated soils, requiring cultivated ground to work efficiently.

1.4.7 Soil sample processing

PCN cysts are extracted from soil by methods of flotation, elutriation or centrifugation. The most widely used flotation method uses the Fenwick can (Fenwick, 1940). Flotation methods have the disadvantage that the soil samples must be dried before use but they are inexpensive and easy to use. The soil is crushed and passed through a sieve of about 4 mm aperture to remove stones and coarse gravel before taking sub-samples, usually of 100, 200 or 500g (Shepherd, 1986). The Fenwick can is subject to some operator error but can extract 97% of the cysts in a sample (Anon, 1991).

The principle of elutriation (separation of different-sized portions by washing) of PCN has been developed from the original design by Seinhorst (1964). A fluidising column for recovery of cyst nematodes is a refinement of the elutriation technique and can be used for general nematode extraction (Trudgill, Evans & Faulkner, 1973). The Wye washer is a modification of the fluidising column and Fenwick can (Winfield, Enfield & Foreman, 1987). The fluidising column of Trudgill *et al.* (1973) was designed for opptimum extraction with 50-100g samples but the Wye washer is of bigger dimensions and can process up to 1 kg of soil (Turner, 1998). The Wye washer can be used for soil without drying and can extract 99% of cysts but uses more water (100 l per sample) (Anon, 1991).

The Schuiling centrifuge which is becoming more widely used, is more rapid and produces a cleaner sample than the other methods. It is virtually free from operator error but expensive to purchase (Anon, 1991). Turner (1998) noted that no one method of PCN extraction is universally ideal for all situations. The method chosen has to consider the predominant soil types, reasons for the extraction and the accuracy. Comparisons of the

Fenwick can and the Schuiling centrifuge found that cysts from soils with a high organic-matter content may be less efficiently extracted with the Schuling centrifuge (Clayden, Turner & Marks, 1985). Comparisons of the Fenwick can and the Wye washer show that the efficiency of extraction of cysts from dry soil is similar but the Wye washer consistently recovers more cysts in moist soil (Turner, 1998).

1.4.8 Plant sampling

Southey (1974) reviewed the methods for detection of PCN. One method of detection is the inspection of growing crops. The inspection of foliage (haulms) for symptoms of attack is of little use. However, during inspection for virus disease, the stunting of plants may be noticed. The presence of PCN can then be confirmed by inspecting the roots.

Wood, Foot, Dale & Barber (1983) discussed the relative efficiency of plant sampling and soil sampling in determining the presence of low PCN infestations in New Zealand. They found that very low populations might be detected by lifting plants and examining the roots while these same populations would not be detected by soil sampling methods. The primary objective of sampling was to detect the presence of PCN in a field as soon as possible after the infestation had occurred so that it could be confined to small patches. A plant sampling method suitable for survey use was developed. Plants were sampled on a 10 row by 10 m grid, giving a sampling intensity of 150-170 plants ha^{-1}. The plant roots were lifted by fork and were examined for developing cysts. This method was compared to the soil sampling methods used in the UK and the Netherlands. At very low populations, the plant sampling method was found theoretically 80-120 times more efficient than the European methods of pre-crop soil sampling. This was supported from data obtained from field experiments comparing plant sampling and soil sampling methods.

1.4.9 GPS mapping of fields

Haydock & Evans (1995) discussed the use of Global Positioning System (GPS) technology in the mapping and management of PCN populations. The mapping of populations of PCN within fields could then be used to spot treat certain areas with nematicides to reduce the cost of nematode control. Evans *et al.* (1998) also investigated the use of GPS technology for mapping populations for modulated applications of nematicide and to assess the consequences of decisions that might have been based on knowledge of the distribution before cropping. They found that where PCN had been undetectable before planting, post-cropping population densities reached up to 50 eggs g^{-1} soil. Nematicides control nematodes more effectively at low population densities but growers only see a return on investment in nematicide application at higher population densities. This leads to hard decisions of whether to use nematicides for short-term economic return in the prevention of yield loss or for the longer-term objective of keeping PCN populations small. Parker (1998) discussed these issues and found that the mapping techniques currently being employed were not accurate enough to justify their use as aids in deciding where to make nematicide applications within fields. There are risks of building up populations in untreated areas of infested fields. However, some mapping techniques may have a role in deciding where to target fumigant use and tracking population multiplication and decline in well-defined areas.

1.5 Control of PCN

In the UK, various methods are used for controlling PCN. These include legislation, crop rotation, resistant cultivars, chemical control and trap cropping. More success has been achieved in controlling *G. rostochiensis* than *G. pallida* for several reasons. The hatching

period of *G. pallida* is longer and nematicide concentrations may have declined to ineffective levels before all of the eggs have hatched (Evans, 1993). In addition, the annual decline rate of *G. pallida* infestations is much lower than that of *G. rostochiensis* (Evans, 1993; Marshall, 1988; Turner & Evans, 1998). In the absence of host crops, *G. pallida* may take as long as 18 years to decline to a non-damaging level compared with 10 years for *G. rostochiensis* (Evans, 1993). With potato growing now concentrated in a smaller area and a concomitant decline in rotation lengths, there has been an increase in levels of *G. pallida* compared to *G. rostochiensis.* The different control methods will be discussed in turn.

1.5.1 Legislation

Since PCN can be spread on soil adhering to tubers, it is crucial to ensure that all seed tubers are free from infestation. In the European Union, certification schemes require that all land must be tested before it can be used to grow a seed crop. If PCN are found, then the growing of a seed crop is prohibited (Evans & Trudgill, 1992).

1.5.2 Crop rotation

A traditionally important control measure is crop rotation. This involves growing non-host crops for a number of years to allow the population to decline naturally. It is particularly effective for PCN since they have a small number of host crops. Winfield (1965) noted that potatoes can be grown once every four or five years in heavy soils, but only once every six or seven years in light and peat soils but there was no evidence that soil type accounts for differences in population levels of PCN. Turner (1996*a*) reported that rate of decline was not affected by soil type, but regular cultivation of infested land appeared to accelerate it. However, Hancock (1988) reported that the hatching rate varies with soil type, although the reasons for this are not known. Hancock (1988) also reported that the hatching rate is

often taken as 30% for modelling purposes and using this figure, a break of 7-8 years is needed for PCN levels to decline to that before the last crop. Various studies have been done on the hatch of PCN (Grainger, 1951; Huijsman, 1961; Cole & Howard, 1962). They found that decline rates ranged from about 30-33% on most soil types in southern England and the Netherlands but could be as low as 18% in the cooler soils of Scotland. Turner (1996*a*) found that decline rates in field soils in Northern Ireland were less than 10% over a 13 year period.

Crop rotation is not necessary for PCN control when growing early varieties in frost-free regions like Ayrshire, Cornwall and Pembrokeshire. The crop is harvested before the females have matured and produced their eggs, so it is possible to grow potatoes repeatedly without populations increasing. (Hominick, 1979).

1.5.3 Biological control

There have been some reports of biological control of PCN *in vitro* or in pots but there is little evidence of effective control in the field (Whitehead, 1998). In pot trials, *Paecilomyces lilacinus* applied to seed potato tubers lessened the numbers of cysts of *G. rostochiensis* in the soil after the plants had been grown, and increased tubers yield six-fold (Gul, Gul & Saeed, 1988). *Verticillium chlamydosporium* was found to infest young females of *G. rostochiensis* but not enough to lessen nematode population density after potatoes had been grown in the pots (Crump & Irving, 1992).

The possibility of controlling PCN using naturally occurring fungal parasites that are involved in their natural control was discussed by Crump (1998). Fungal parasites of the female stage of the nematode are likely to be the most effective at reducing nematode

populations but, so far, none has given significant control when tested in the field. Many of these fungi are saprophytic or root colonising species and, although some strains can parasitise nematodes, this is not their main form of survival. Under field conditions, not enough nematodes are killed to have an effect on multiplication (Crump, 1998).

Perry, Twomey & Rolfe (2000) studied the effects of DiTera® (Valent BioSciences Corp.) on aspects of the life cycle of *G. rostochiensis.* DiTera® is a product of biological origin, obtained by submerged fermentation of a strain of the soil hyphomycete fungus *Myrothecium verrucaria.* It has been demonstrated in laboratory bioassays to adversely affect hatch, movement and sensory perception of J2s of *G. rostochiensis.* In field conditions, interference with orientation to host roots could result in random movement and a probable decrease in the numbers of J2s successfully invading, although this work has yet to be done.

1.5.4 Trap cropping

Trap cropping can lessen the soil infestation of PCN if potatoes are grown long enough to stimulate hatch of the nematodes and invasion of the potato roots and then are lifted and removed from the infested area (Whitehead & Turner, 1998). The plants must be lifted before the females are fertilised, so that no new eggs are left in the soil. The very tolerant cultivar Cara grown in full ridges for about 6 weeks in heavily infested soil decreased *G. pallida* by 75% or more (Whitehead *et al.*, 1994). The technique works best on heavily infested sites and some other commercial late-planted crop could follow if it was done in spring or early summer.

1.5.5 Resistant cultivars

A third important strategy is the use of resistant cultivars, which provides a cheaper alternative to chemicals. There are many commercial cultivars of potato that are resistant to *G. rostochiensis* (Evans & Haydock, 1990). This resistance is stable and complete and is conferred by a single dominant gene (H_1). However, it is only effective against two pathotypes of *G. rostochiensis* and is not effective against *G. pallida*, with the consequence that, when used in fields containing both species, *G. pallida* will rapidly increase while *G. rostochiensis* declines. The most widely grown ware cultivar Maris Piper has the H_1 resistance gene. Huijsman (1961) reported that growing resistant cultivars decreased infestations more than growing non-host crops, but the species and pathotype he used are unknown. However, after growing resistant cultivars for six years, an infestation was still present.

Cole & Howard (1962) reported field experiments in which resistant cultivars were grown continuously for six years on an infested site. The site contained populations of what was then described as biotype A and biotype B. This work was done before the two PCN species had been recognised by Stone (1972) and biotype A is now recognised as being *G. rostochiensis* and biotype B as *G. pallida*. They found that continuous growing of resistant cultivars changed the population from almost entirely *G. rostochiensis* to largely *G. pallida* within six years. With potatoes grown every four or five years, such a change would take at least 20 years. Nevertheless, resistant cultivars have been available for over 30 years and *G. pallida* is now the major problem in many areas.

The search for resistance to *G. pallida* has been less successful with no major gene effective against many populations having yet been found. Plant breeders have had to use

polygenic resistance from wild potato species. Introducing this into commercial cultivars is a lengthy procedure and relatively few cultivars are available, none of which offers more than partial control. This lack of totally effective resistance contributes significantly to the pest status of *G. pallida*. Whitehead, Tite, Fraser & Nichols (1984) noted that in Britain the widespread growth of Maris Piper potatoes (resistant to *G. rostochiensis* but not *G. pallida*) has increased the incidence of the latter.

Finlay, Dale & de Scurrah (1998) commented that little is known about how the different sources of PCN resistance act to prevent nematode increase. Hoopes, Anderson & Mai (1978) noted that limited nematode development on resistant plants is caused by poor establishment of feeding sites. Although juvenile nematodes were able to penetrate roots of resistant hosts and initiate syncytial development as in susceptible hosts, these syncytia ultimately degenerated. Phillips, Forrest & Farrer (1982) investigated the invasion and development of *G. pallida* in the susceptible cultivar Pentland Crown and in two *Solanum* genotypes with resistance derived from *S. vernei*. They found that the number of juveniles within the roots of Pentland Crown and the less resistant genotype were similar, while the highly resistant genotype had significantly lower numbers. These differences may have been as a result of different hatching patterns. The development of the juveniles was also substantially retarded in the most resistant genotype and a higher ratio of males to females was observed in the resistant genotype. They suggested that reduced invasion and establishment can contribute to resistance to PCN.

Turner & Stone (1984) studied the development of PCN in roots of resistant plants. Comparisons were made between development in plants with the major gene H_1 derived from *Solanum tuberosum* ssp. *andigena*, plants with polygenic resistance derived from *S.*

vernei and a susceptible cultivar. They found the numbers of juveniles invading root systems were not clearly related to resistance but in all combinations of potato and nematode where resistance was found, there was a decrease in the rate of nematode development which varied in degree but occurred with both types of resistance. They concluded that this appears to be the main effect of resistance on nematode multiplication.

Forrest, Trudgill & Cotes (1986) studied the invasion and emergence of juveniles *G. rostochiensis* and *G. pallida* in roots of susceptible cultivars (Pentland Crown and Katahdin) and resistant potato cultivars (Maris Piper and Rosa) with gene H_1. They found that the invasion rates were similar for both species and all cultivars. However, it was found that more second stage juveniles re-emerged from the roots for Maris Piper and Rosa. Forrest, Robertson & Trudgill (1984) reported that the binding of non-protein lectins to PCN juveniles, suggesting that certain sugar moieties on the nematode cuticle may have a role in the re-emergence of nematodes from roots of resistant genotypes.

Mullin & Brodie (1988*a*) found that the numbers of J2 that invaded a susceptible and a resistant cultivar did not differ significantly. They found that significantly more juveniles egressed from the root of the resistant cultivar. Significantly fewer males developed in and emerged from resistant host roots, relative to susceptible ones and any females that do develop have reduced fecundity (Mullin & Brodie, 1988*b*). Plants carrying the H_1 gene were also found to stimulate PCN hatch and are invaded to similar extent to plants without the gene (Rawsthorne & Brodie, 1986).

Williams (1958) and Turner (1980) noticed a reduction in the efficacy of root leachate to stimulate larvae to hatch for *S. vernei*. Finlay, Dale & de Scurrah (1998) reported that there

is an important interaction between the hatching patterns of nematode populations and potato genotypes. It was reported that there were important qualitative and quantitative differences in hatching patterns between *G. pallida* populations. Hatch inhibition thus may be an important ancillary resistance mechanism (S J Turner, unpublished data).

A review by Evans & Haydock (1990) thought that it was unlikely that cultivars with the H_1 resistance gene stimulate fewer juveniles to hatch than non-resistant cultivars but the reverse may be true for potato clones with resistance derived from *S. vernei*. Evans & Haydock (1990) also noted that there is variation between cultivars to stimulate hatch but this is usually quite small.

1.5.6 Chemical control

Chemicals are widely used in the UK to control PCN. Two groups of chemicals are used for PCN control: specific nematicides and broad spectrum soil sterilants.

1.5.6.1 Nematicides

Most non-fumigant nematicides are organophosphates and carbamates, which are powerful anticholinesterase inhibitors but are non-phytotoxic and so can be applied at planting time (Whitehead, 1998). They act as nematostats, which paralyse rather than kill the nematodes, except when used in exceptionally large amounts (Whitehead & Turner, 1998). They are effective at dosages smaller than those needed for control by fumigant nematicides. The organophosphates are cumulative mammalian poisons and are now seldom used for PCN control. The most commonly used nematicides in the UK are the carbamates aldicarb (Temik) and oxamyl (Vydate) (Evans & Haydock, 2000). They are used in granular form with the granules mixed into the top 15 cm of the soil immediately before planting.

Control of *G. pallida* by aldicarb or oxamyl may be more variable than control of *G. rostochiensis* (Whitehead & Turner, 1998). *Globodera pallida* was sometimes controlled as well as *G. rostochiensis* but often it was not (Whitehead *et al.*, 1984). Whitehead (1992) suggests that inadequate control of *G. pallida* increase on susceptible potatoes by granular nematicides is primarily due to the slow hatch of *G. pallida* continuing for periods longer than the persistence of the nematicide.

The effectiveness of nematicides for the prevention of yield loss was studied by Brown (1983) and Trudgill (1986), who both found that yield loss due to PCN damage could be prevented by the application of aldicarb or oxamyl. These chemicals are not fully effective on their own in eliminating the problem. They offer protection against damage and prevent yield loss but do not completely prevent invasion of the juveniles, which can multiply greatly at low densities leaving the soil heavily infested (Phillips & Trudgill, 1998).

1.5.6.2 Fumigant nematicides

Soil fumigation was developed over a century ago in France, where carbon disulphide (CS_2) was used to control phylloxera on grape roots (Radewald, McKenry, Roberts & Westerdahl, 1987). After World War I, it was found that chloropicrin (used in the war as a tear gas) was effective at killing nematodes and insects when used as a soil fumigant.

Soil sterilants may be fumigants *per se* or decompose in the soil into fumigant breakdown products. Soil sterilants kill a wide range of soil-borne organisms and do so by fumigation. The chemical is injected into the soil where it volatilises. The gas gradually escapes

through the surface of the soil which must be sealed to keep the gas in contact with the nematodes long enough to be effective (Whitehead & Turner, 1998).

Carter (1943) discovered a chemical nematicide that triggered the rapid growth of nematology. This was the soil fumigant dichloropropene-dichloropropane which was produced and marketed by the Shell Chemical Corporation as D-D (Webster, 1998). The major constituents of D-D are (Z)-1,3-dichloropropene, (E)-1,3-dichloropropene and 1,2-dichloropropane, which constitute approximately 28, 26 and 23% respectively (Roberts & Stoydin, 1976). The soil fumigant Telone II is comprised of 94% 1,3-dichloropropene (1,3-D), of which there are equal ratios of the cis- and trans- forms of the molecule (Ou *et al.*, 1995).

Ethylene dibromide (EDB) was more effective than the D-D mixture and, in the late 1940s, DBCP (1,2-dibromo-3-dichloropropane) was patented as a soil fumigant and plant growth stimulator (Radewald *et al.*, 1987). DBCP is almost unique as a soil fumigant in having low phytotoxicity and was marketed as Fumazone (Dow Chemical Company) and Nemagon (Shell Chemical Company). Methyl bromide also came into use at much the same time and this controls most soil-borne pests (Radewald *et al.*, 1987).

1.5.6.3 Fumigation with 1,3-D

The area treated with fumigants in the UK is increasing and, in 1998, about 1,250 ha were treated at a cost of approximately £600 000 (Parker, 1999). Many experiments have been done to assess the use of 1-3-D and dichloropropene-dichloropropane mixtures for PCN control.

Whitehead, Fraser & Storey (1972*a*) investigated the use of D-D for PCN control and its effect on potato yields. They applied D-D in spring and then covered the ground with polyethylene sheeting. They found that D-D increased the yield of tubers but also increased the number of cysts in the soil. Whitehead, Tite, Fraser & French (1972*b*) also compared D-D and aldicarb in peaty loam soil, using the cultivars King Edward and Maris Piper. The D-D was injected 15 cm deep into the soil at different rates. It was found that large amounts of D-D, which were needed to control PCN in the first year, did not control PCN in the second year. The yield of King Edward was increased in both years. It was also found that rotary cultivation of the soil after injection increased the effect of D-D, probably by improving its distribution. The soil was then covered with plastic sheeting.

Whitehead, Tite, Fraser & French (1973*a*) treated potato ridges with D-D and found that it escaped too rapidly from sandy soil and was adsorbed by the peat in peaty loam soil. In sandy clay soil, potato yields were increased more by treating the potato ridge soil in the spring before planting rather than treating the topsoil during the preceding autumn. This was probably due to the greater concentration of nematicide surrounding the young potato plants giving them greater protection. It was also thought that D-D may still have some effect after planting. In the peaty loam soil, D-D was not as effective as aldicarb at decreasing the number of infective juveniles in the soil at planting.

Whitehead, Tite, Fraser & French (1973*b*) looked at the effects of the soil fumigants D-D and dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) on PCN when applied to potato ridges in spring. Large amounts of D-D applied to the top-soil in autumn or to the ridges in spring were less effective in controlling PCN or increasing yield than dazomet. Whitehead, Tite, Fraser & French (1973*c*) applied a 1,3-D mixture and dazomet

in different ways to look at PCN control. They found that when large amounts of 1,3-D are applied in the spring, the topsoil will still contain phytotoxic residues after several weeks which could delay planting and lead to a decrease in yield. Experiments were done applying a 1,3-D mixture in autumn and in spring. A 1,3-D mixture was injected 25 cm deep and in peaty loam soil at 20-40 cm deep there were as many or more nematodes after harvest in treated as in untreated plots. There were few nematodes 40-60 cm deep and the soil 20-40 cm deep was much less infested than soil from 0-20 cm deep. However, dazomet controlled PCN better than a 1,3-D mixture and it was thought that this was partly due to the 1,3-D mixture escaping from the soil surface even though it had been rolled. In this case, a 1,3-D mixture was as effective when applied in the autumn as in the spring. Treating in the autumn ensured that planting was not delayed.

Whitehead, Fraser, French & Wright (1975) studied control of PCN by fumigants on tomatoes grown under glass. Plots treated with dazomet and injected with a 1,3-D mixture were covered by polythene sheets 0.1 mm thick buried 20 cm deep at the edge of the plots. It was found that injecting a 1,3-D mixture 23 cm into the soil after adding dazomet increased nematode control in the top 20 cm of soil compared with treating by dazomet alone. Further work by Whitehead, Fraser & French (1979) used a 1,3-D mixture at a rate of 448 kg ha^{-1}, injected 23 cm deep and covered with polythene sheets 0.1 mm thick buried 15 cm deep at the edge of the plots. A 1,3-D mixture with or without oxamyl controlled PCN satisfactorily and greatly increased yields. Steaming was also tried and involved covering plots with polythene and steaming for 4 hours. This did not control PCN in soil 20-40 cm deep as well as the fumigants.

Whitehead, Tite, Fraser & French (1980) used a 1,3-D mixture in three course rotations: potatoes - sugar beet - spring barley - potatoes. The fumigants were applied either before or before and after the first potato crop. It was found that a 1,3-D mixture lessened the numbers of juveniles invading potato roots but, after potatoes had been grown, the treated plots were as infested as untreated plots. Whitehead *et al.* (1980) noted that increases in yield were not a reliable guide to nematode control. A 1,3-D mixture kills the juveniles in the cyst but, because it also kills soil bacteria, the dead juveniles can be preserved for months, making it difficult to assess kill. The different modes of action of fumigant and non-fumigant nematicides mean that comparison of nematode control is difficult between different nematicide treatments. Whitehead *et al.* (1973*b*) were able to determine the percentage of dead eggs in the cyst by staining with New Blue R (Shepherd, 1962) several months after fumigation. However, this is time consuming and was not always used.

Whitehead *et al.* (1980) assessed PCN levels in an experiment involving 1,3-D by only counting total embryonated eggs and juveniles extracted from cysts from air-dried soil samples. This is satisfactory for cysts from untreated or non-fumigant treated soil. However, after harvest there appeared to be more nematodes in plots which had received a 1,3-D mixture plus oxamyl than in plots treated with a 1,3-D mixture alone because the counts included eggs which were dead but preserved by 1,3-D. The egg counts after two subsequent crops of sugar beet and barley gave more reliable comparisons of degree of control.

Whitehead & Nichols (1992*a*) reported the control of *G. rostochiensis* by nematicides applied either once or twice in rotation. The fumigant 1,3-D was applied in the autumn followed by a spring application of a granular nematicide. 1,3-D was injected 20 cm deep

into the soil at a rate of 224 kg ha^{-1}. It was found that plots treated with 1,3-D and aldicarb or oxamyl at 2.8 kg ha^{-1} had higher yields than those treated with only aldicarb or oxamyl at 2.8 kg ha^{-1}. However, plots treated with 1,3-D did have larger Pf/Pi values since the counts were for total egg counts and would therefore count those eggs which had been killed but also preserved by 1,3-D. After three arable crops, the numbers of eggs in plots which were treated with 1,3-D were no more than in those plots which received only a granular nematicide. It seems likely that the eggs that were killed by the 1,3-D had finally decayed.

Whitehead *et al.* (1994) looked at the control of *G. pallida* by chemical and cultural methods in different soils. One of these experiments measured the effects of 1,3-D on tuber yield and *G. pallida* multiplication in comparison with other nematicides. Plots fumigated with 1,3-D had 62, 46 and 19% of the viable eggs killed at each of the three trial sites. In three plots at one trial site, 81% of the eggs in the top 15 cm and 91% of eggs in the 15-30 cm layer of soil had been killed. In three plots at another trial site, 0% and 32% of viable eggs had been killed in the 0-15 cm and 15-30 cm layers of the soil. The 1,3-D reduced the number of viable eggs at the two trial sites with silty loam and sandy loam soil but had much less effect in the peaty loam soil. Tuber yields were significantly increased by 1,3-D at all three trial sites. However, not all of this increase could be due to the nematicidal effect of 1,3-D and part of it may have been as a result of inhibition of nitrifying bacteria, making more N (in ammonium form) available to the plants. As already mentioned, yields of up to of 90 t ha^{-1} were achieved by Gunn (1978). These yields are uneconomic due to the law of diminishing returns since maximum profit is achieved at lower input levels and lower yields (Evans & Haydock, 1990). Whitehead *et al.* (1994) also noted that the availability of trace elements may also have been altered which may have contributed to

the yield increase. Whitehead *et al.* (1994) concluded that, although 1,3-D increased tuber yields more than granular nematicides, it did not lessen *G. pallida* multiplication. Since multiplication is density dependent, and there is greater multiplication at lower population densities (Turner & Evans, 1998), then the use of 1,3-D may increase the rate of *G. pallida* multiplication where a susceptible cultivar is grown.

1.5.6.4 Surface sealing to improve control by fumigation

The effectiveness of the fumigant depends on it remaining in contact with the nematode long enough to kill it (Alphey, 1980). Liquid fumigants are injected into the soil, where they form a gas which diffuses through the pore spaces and can escape into the atmosphere when it reaches the soil surface. The fumigants may not stay in the soil long enough near the soil surface to kill the nematodes. Peters (1955) reported that nematodes were practically eliminated at depths between 5 and 25 cm but that the kill is unsatisfactory in the surface layer. Laboratory experiments indicated that there is a considerable loss of fumigant from the surface layer of soil that could be reduced by using a water seal or a non-gas-tight seal of foil.

Methods that can be used to seal the soil surface include: covering the soil surface with gas-tight sheeting, wetting the surface or rolling. For PCN control, the liquid fumigant 1,3-D is injected 20 cm deep into the soil by a Rumptstad Combiject (Rumptstad, Haringvliet, The Netherlands), which has a powered roller to smear the soil surface and so seal it after injection. The soil type largely determines the quality of the seal and certain soils do not seal well, but the moisture content of the soil is also important. The alternative of using gas tight sheeting is expensive and it is difficult to apply. Wetting is difficult to do practically and requires a lot of water, but modern irrigation equipment may make it easier. Alphey

(1980) tested the efficacy of 1,3-D with different methods of soil sealing including: rolling, sealing by rolling with a resin spray and raking dazomet into the surface before rolling. No significant improvements were found in sprayed treatments fumigated with the same rate of 1,3-D as unsprayed controls. It was thought that this was due to the resin not being thick enough or due to its cracking after drying out, thus letting the fumigant escape. The use of dazomet with 1,3-D was only partly effective but this may have been because the dazomet was not incorporated properly.

Van Wambeke (1990) reported that there has been little study on the use of gas-tight soil mulches with 1,3-D. Both (Z)- and (E)-isomers of 1,3-D are present in commercial fumigants and the diffusion of both of them through various mulches was investigated in the laboratory using gas chromatography. Various plastic films were compared with polymer sprays and biodegradable plastics. It was found that the two isomers diffuse at different rates with the (E)-isomer having a lower rate of loss. The experiments using the biodegradable film showed that the permeability of the plastic film is affected by temperature. Van Wambeke (1990) reported some potential for polymer emulsions and indicated that water applied to some finely crumbled soil surfaces may reduce fumigant losses by up to 50%.

McKenry *et al.* (1978) discussed the movement and toxicity of soil fumigants including 1,3-D. They found that the condition of the field prior to fumigating is an important factor in the control that will be achieved. Soils that are wet (below -60 centibars moisture tension), cold (below 10^0C), very warm (above 25^0C) and with a high organic matter content (above 2.5%) are the most difficult with which to get good results. Other factors

reducing fumigant efficiency are the presence of roots, dry fine-textured soils that form clods, and high rainfall in highly permeable soils.

1.5.7 Integrated control

Integrated control of PCN involves the use of rotations, resistant cultivars and nematicides (Trudgill *et al.,* 1987; Alphey, Phillips & Trudgill, 1988). It aims not only to reduce yield loss but also to reduce the population density attacking the next crop while reducing effects like the selection of virulent pathotypes (Roberts, 1993). The aim is not to eliminate the pest but to keep population densities within acceptable limits using several control measures (Evans, 1993). The control measures may be used in consecutive years and in combination with each other. Control measures are given multiplication factors (F) which can be multiplied together to project the population density in the final year of the cropping cycle. This can be expressed algebraically:

$$Pf = Pi(F_1)^a(F_2)^b(F_3)^c \ldots\ldots(F_n)^n$$

where Pf and Pi are the final and initial population densities of PCN, F_1 .....F_n are various control measures and the exponents a,b,c *etc,* denote the number of years for which each is practised (Evans, 1993). An example of this for control of *G. rostochiensis* (giving a Pf of 38 from a Pi of 100) is to grow three non-host crops in rotation with one resistant crop and one non-resistant crop treated with nematicide.

The control of *G. pallida* is much more difficult than that of *G. rostochiensis* because it persists longer, is less susceptible to nematicides and there are no fully resistant cultivars. Integrated control therefore includes the currently available control strategies but hopefully will include new strategies as they are developed, such as artificial hatching agents and biological control agents.

1.6 Vertical distribution of PCN

Potato plants are shallow rooted with the roots usually not more than 40-50 cm deep. However, roots can extend to 1 m if there is no obstruction in the soil profile (Brodie *et al.*, 1993). Potato cyst nematodes need to be able to migrate to and invade potato roots in order to reproduce. Peters (1953) investigated the vertical distribution of cysts in soil. The vertical migration of PCN was investigated by growing a potato plant in a wooden box of soil which was free from cysts apart from those added in a thin layer at a known depth. It was found that juveniles can migrate both downwards and upwards over distances of about 15 to 20 cm. In the layer of soil furthest from the inoculum (20 to 25 cm away), only a very small number of cysts formed (less than 1% of the total).

Whitehead *et al.* (1973*c*) measured the control of PCN in sandy, peaty and silt loam soils by Telone (1,2-dichloropropane, 1,3-dichloropropene mixture) applied in different ways. In silt loam soil 20-40 cm deep there were at least as many nematodes in fumigated as unfumigated plots. In untreated plots there were fewer PCN after harvest in soil 20-40 cm deep than in soil 0-20 cm deep, and soil 40-60 cm deep was sparsely populated with PCN. In sandy loam soil it was also found that soil in the top 20 cm contained more PCN than soil 20-40 cm deep and that soil 40-60 cm deep contained the least PCN. Whitehead (1977) collected cores from 35 fields in England heavily infested with PCN, beet cyst nematode or pea cyst nematode and found that PCN were often as numerous in soil 20-40 cm deep as in soil 0-20 cm deep. Below 40 cm, the soils were very lightly infested.

Further work by Whitehead *et al.* (1980) on control of PCN showed that, after Pentland Crown potatoes, very few eggs were found in soil 40-60 cm deep and few were found 20-

40 cm deep. However, in the top 20 cm the number of nematodes increased greatly after Pentland Crown potatoes had been grown. This may reflect the very shallow root system found in this cultivar (Evans & Haydock, 1990).

1.7 The effects of fumigant nematicides on soil nitrification

Fumigant nematicides can affect microorganisms involved with soil fertility. Martin & Pratt (1958) noted that nitrifiers appeared to be more sensitive to soil fumigants than ammonifiers and that their activity might be reduced for several weeks to several months. In contrast, the organisms that release ammonium nitrogen from organic nitrogenous compounds quickly increase in numbers following treatment. The net result of this is that ammonium nitrogen accumulates in the soil and, since any ammonia added in fertiliser will remain as ammonium, concentrations of ammonium can reach very high levels- so much so that ammonium sensitive plants may be injured.

Many studies have indicated that soil fumigants retard or inhibit nitrification of ammonium nitrogen: the fumigants that are used as nematicides have exactly this effect (Wolcott, Liao & Kirkwood, 1967; Jenkinson & Powlson, 1970; Marks, Elliot & Tu, 1972; Tu, 1972). Elliot, Marks & Tu (1974) found increased levels of NH_4^+-N and reduced levels of NO_3^--N in soils that had been fumigated. Lower levels of NO_3^- were found in fumigated soils by Marks *et al.* (1972).

Wolcott, Liao & Kirkwood (1967) found that, after fumigation with dichloropropene, the pattern of nitrate disappearance and accumulation reflected a simple commensalistic interaction between the autotrophic nitrifiers and heterotrophic groups using nitrate for respiration and/or growth. After a period of nitrate disappearance, nitrate began to

accumulate abruptly. The rate of accumulation was immediately equal to that observed earlier in unfumigated soil where no initial disappearance of nitrate occurred. Williams & Salt (1970) also found that mineralised nitrogen increased after treatment with D-D (1,2-dichloropropane, 1,3-dichloropropene mixture). Most of the increase was NH_4^+ and very little NO_3^-.

Although fumigants are used primarily to control pests and diseases, it has been reported that soil fumigation enhanced crop growth even in the absence of serious root disease (Rovira, 1976; Jenkinson & Powlson, 1970). Rovira (1976) found an increase in nitrogen uptake in wheat after fumigation with chloropicrin and concluded that this was partly due to an enhancement of ammonium release and inhibition of nitrification with a consequent increase in uptake by the plants. The NH_4^+-N in the soil is leached less rapidly and cannot be lost by denitrification. Jenkinson & Powlson (1970) found a declining response to repeated fumigation: the flush of mineral nitrogen accompanying a second fumigation is less than from the first fumigation. When nitrogen is limiting growth, a second fumigation will be less effective than the first in increasing yield. Even when the second fumigation is five years after the first, the effect of the first treatment will still persist and less nitrogen will be mineralised than if the soil had never been fumigated.

Tu (1993) studied the effects of fumigant nematicides on microflora and nitrification in tobacco soil. Nitrification was inhibited six days after treatment with Vorlex (1,3-dichloroprene and related C_3 hydrocarbons - 80%; methyl isothiocyanate - 20%) and one day after treatment with Telone II (1,3-dichloropropene). However, after three weeks, the fungal populations had recovered to a level equal to that found in the control.

The effect of dichloropropene on microbial activities in soil was studied by Tu (1994). A significant stimulatory effect was observed on activities of soil ammonifiers one week after treatment with dichloropropene. The formation of nitrite and nitrate from nitrogen compounds is the second stage in the nitrogen cycle in soil. Nitrifying microorganisms produce nitrate, which is the major nitrogen source assimilated by higher plants. This reaction is mainly carried out by *Nitrosomonas* spp. and *Nitrobacter* spp. Nitrification was also reduced by dichloropropene but it recovered after three weeks, which suggests that nitrifying organisms recover and nitrification proceeds in a normal fashion.

The process of microbial denitrification is the reduction of NO_3^- and NO_2^- into nitrous oxide (N_2O) or nitrogen gas (N_2), which is lost from the soil into the atmosphere and is therefore lost to microorganisms and plants. The microorganisms which take part in microbial denitrification are mainly *Pseudomonas* spp., *Bacillus* spp. and *Paracoccus* spp. (Alexander, 1977). Tu (1994) found that dichloropropene was non-toxic to denitrifying microorganisms and a stimulatory effect was observed two weeks after treatment, which may suggest that the metabolites of dichloropropene can act as a substrate for certain microorganisms under certain environmental conditions.

The effects of nematicides on soil nitrification were studied by Tu (1996), who noted that the treatment of soil with chemicals might result in changes in populations of microorganisms related to soil fertility. Telone C (1,3-dichloropropene and related C_3 hydrocarbons - 85%; chloropicrin - 15%) decreased nitrification activity. Treatment with Vorlex (1,3-dichloroprone and related C_3 hydrocarbons - 80%; methyl isothiocyanate - 20%) also decreased nitrification activity after two weeks.

Nira, Hashimoto, Matsuzaki & Nishimune (1996) studied nitrogen transformations and availability in soils with application of fumigants. It was found that the application of D-D (1,2-dichloropropane, 1,3-dichloropropene mixture) increased the nitrogen availability in soil in spite of a decrease in the gross rates of nitrogen transformations. D-D decreased the rate of nitrogen transformation, which was attributed to an inhibitory effect on microbial activities. The recovery of the soil was not complete for up to nine months after fumigation in some plots. The content of ammonium in soil increased and nitrification was inhibited by the application of D-D. There was an increase in the net rate of nitrogen mineralisation, which contributes to nitrogen uptake by crops. The accumulation of ammonium in soil, the inhibition of nitrification and the increase in nitrogen mineralisation may contribute to nitrogen uptake by crops.

The results of the research reviewed above suggest that fumigant nematicides such as 1,3-D can have an effect on crops in the absence of attack by pests or diseases by their effects on soil microorganisms. The effects on the microorganisms that are involved in the nitrogen cycle have been shown to increase the amount of nitrogen available to the plant, which can increase yields if nitrogen is the limiting factor.

1.8 The effect of 1,3-D on the incidence of *Rhizoctonia solani*

The soil fumigant 1,3-D is a broad spectrum soil sterilant that affects many organisms in the soil. In addition to controlling PCN, a possible effect of using 1,3-D is the control of the fungus *Rhizoctonia solani* Kühn, a common disease of potatoes, which is also known as Black scurf and Stem canker.

Seed-tuberborne and soilborne inocula of *R. solani* have both been implicated in the epidemiology of stem canker on potatoes (Gilligan, Simons & Hide, 1996). Hide & Read (1991) reported that the severity of stem canker increased with increasing frequency of previous potato crops, which suggests that soil-borne inoculum of *R. solani* had increased. Adams & Hide (1980) found that there are significant relationships between incidence of disease on seed and that of harvested or stored tubers, indicating that the seed tubers were a source of inoculum.

Simons & Gilligan (1997) reported that there are contradictory reports of the effects of growing conditions on the development of stem canker from planting onwards. Some reports indicate a close association between the severity of black scurf on seed tubers at planting and the development of stem canker (Gudmestad, Zink & Huguelet, 1979; Chand & Logan, 1982; Read, Hide, Firmager & Hall, 1989) while others indicate a more variable relationship (Hide, Hirst & Stedman, 1973; Adams & Hide, 1980). Adams, Hide & Lapwood (1980) reported that environmental conditions at planting may influence development of stem canker more than the severity of tuber-borne inoculum on seed tubers.

The symptoms of *R. solani* infection are lesions on stems and stolons, and sclerotia on the tubers and roots. During the vegetative period, a white collar of mycelium with basidia may be formed around the stem base (Hofman & Jongebloed, 1988). *Rhizoctonia solani* also causes delayed emergence and leaf rolling (Harris, 1992). Plants are not killed but crop development is delayed and tuber initiation and bulking are further delayed by the infection and pruning of stolons (Hide, Read & Sandison, 1985*a*). Infection of shoots by *R.*

solani soon after planting delays stem emergence and development of foliage (Hide, Read & Sandison, 1985*b*).

When plants are affected by a combination of nematodes and pathogenic fungi, they can often suffer damage greater than the sum of that caused by each of the organisms singly (Harris, 1992). The combination of the symptoms caused by *R. solani* together with PCN was called "potato sickness" by Morgan (1926) and more recently by Grainger & Clark (1963) and Dunn & Hughes (1967). It would therefore be highly beneficial if a single chemical treatment could control both *R. solani* and PCN since the benefits could also be greater than the reduction in damage caused by each organism singly.

Previous studies have looked at the effect of granular nematicides on *R. solani.* An increased infection of potatoes and beet by *R. solani* was observed after application of the granular nematicides aldicarb, oxamyl and ethoprophos (Hide & Corbett, 1974; Leach & Frank, 1982; Ruppel & Hecker, 1982; Scholte, 1987). Scholte (1987) studied the effect of crop rotation and granular nematicides on the incidence of *R. solani* in potatoes on marine clay and sandy soil and found that application of aldicarb, oxamyl and ethoprophos resulted in a marked increase of infection of stems and stolons. In some cases, double the levels of infection were observed but the reasons for this increase were unexplained.

Hofman & Bollen (1987) also studied the effects of granular nematicides on growth and microbial antagonism to *R. solani.* They observed no negative effects of the nematicides on the microbial antagonism to *R. solani* or on growth of *R. solani.* Taken together, these results suggest that the nematicides increase the susceptibility of the potato plant to *R. solani* or suppress the activity of mycophagous soil fungi.

There is less known about the effects of 1,3-D on *R. solani.* However, there has been some work done on the control of other soilborne fungi by 1,3-D. The control of soilborne pathogenic fungi in fields of sweet onions by 1,3-D was studied by Sumner *et al.* (1997). They found that 1,3-D was ineffective at controlling a range of soilborne pathogenic fungi including *R. solani.*

Studies have been done comparing the toxicity of 1,3-D to *Phytophthora citrophthora* (R.E.Sm. & E.H.Sm.) Leonian and *Phytophthora parasitica* Dastur with that of the citrus nematode *Tylenchulus semipenetrans* (Cobb) (Baines *et al.,* 1966; Baines, Klotz & Dewolfe, 1977). It was found that 1,3-D was toxic to both types of organisms but was more toxic to the nematodes than the fungi. The fungus *Sclerotium cepivorum,* which causes white rot disease in bulb onions, was controlled by using 1,3-D (Davies, 1990).

The effect of 1,3-D on black dot disease of potatoes caused by the fungus *Colletotrichum coccodes* (Wallr.) Hughes was studied by Read & Hide (1995). In addition, they measured *R. solani* levels. An assessment made when the crop was growing revealed that 1,3-D did not decrease black dot but increased the prevalence of *R. solani* on eye tissue plugs. Similarly, at harvest, there was no effect of the treatment on the amount of black dot but black scurf was increased. Stevenson, Green & Bergeson (1976) also found that 1,3-D failed to control *C. coccodes.*

Altman & Fitzgerald (1960) found that in a field with a high incidence of *Rhizoctonia* and that had been fumigated the previous year, areas that had been fumigated developed less *Rhizoctonia* infection. Tu (1993) studied the effects of nematicides on microflora and

nitrification. He found that 1,3-D had inhibitory effects on soil populations one day after treatment. However, after three weeks, fungal populations recovered to the level found in the control.

Therefore, there is some evidence for the effectiveness of 1,3-D for the control soilborne fungi, but its use for the control of *R. solani* on potatoes at rates used for PCN control is not yet fully understood.

1.9 The effect of 1,3-D on the germination and growth of weed seeds

A MAFF pesticide usage survey found that herbicides were applied to 98.6% of ware potato crops and that ware potato crops received on average 1.7 herbicide sprays (Thomas, Garthwaite & Banham, 1997). Harris (1992) noted that there are four main types of herbicide used for weed control in potatoes:

1. pre-planting- the herbicide is applied before the potatoes are planted
2. contact pre-emergence- the herbicide is applied to the foliage of emerged weed seedlings after the crop has planted but before it has emerged
3. soil acting pre-emergence- the herbicide is applied after planting but before both potatoes and weeds have emerged
4. post-emergence treatments- the herbicide is applied after both the crop and weeds have emerged

The MAFF pesticide usage survey found that the most extensively used herbicide formulations, principally for general weed control, were paraquat (Gramoxone 100, Zeneca) (20% of the treated area), metribuzin (Sencorex WG, Bayer) (15%), linuron (Afalon, Aventis) (12%), and diquat/paraquat (PDQ, Zeneca) (11%). The results also show

that there was an average of 3.1 active substances applied including repeat applications of the same active substance (Thomas, Garthwaite & Banham, 1997).

The cost of a spray programme involving several active ingredients varies depending on the cost of the individual products used. The typical cost of some of the herbicides applied to potatoes are listed in the Farm Management Pocketbook (Nix, 2000). The pre-emergence herbicide monolinuron (Arresin, Aventis) costs between £31-47 ha^{-1} while the pre- and post-emergence herbicides metribuzin (Sencorex WG, Bayer; Lexone 70DF) cost £25-50 ha^{-1} and terbutryn + terbuthylazine (Opogard, Novartis) cost £22-32 ha^{-1} (Nix, 2000). Therefore, the cost of a spray programme for the control of weeds in a potato crop could be in excess of £100 ha^{-1} if around 3 active substances were used.

Soil fumigants containing 1,3-D have been used for many years to control nematodes. However, the use of 1,3-D has potential benefits for weed control since it is toxic to most plants and therefore can have a herbicidal activity when used as a soil fumigant. There have been some reports on the incidental control of weed problems and these are discussed below.

The use of D-D (1,2-dichloropropane, 1,3-dichloropropene) for the control of weeds was studied by Altman & Fitzgerald (1960). They found that autumn fumigation reduced the weed population in the following crop of sugar beet. They observed reductions in bindweed, grasses and pigweed from naturally occurring populations on the experimental site. The weed reduction may have been due in part to a direct effect on the weed seed and in part to shading from the more vigorous growth of beet foliage in treated plots, which caused a reduction in weed seed germination and growth.

Turner, Greathead & Welch (1974) carried out an experiment for the control of annual weed seeds with 1,3-D. In untreated areas of the field, a severe weed problem developed while the areas treated with 1,3-D were almost weed free. The main weeds in untreated plots were common sow thistle (*Sonchus oleraceus*), common groundsel (*Senecio vulgaris*), nightshade (*Solanum* sp.) and cheeseweed (*Malva parviflora*). They gave 1,3-D (at a rate of 225 l product ha^{-1}) a score of 7 on a scale of 0-10 for annual weed control, with 10 = perfect control.

A study to evaluate the use of 1,3-D for the control of the weed field horsetail (*Equisetum arvense*) was done by Coupland & Peabody (1980). The extensive rhizome system of this weed, which can penetrate deeply into the soil, is the main factor contributing to its success. The results of their experiments suggested that 1,3-D was an effective treatment for the control of field horsetail. They found that the fumigated plots were completely weed-free for almost two months. When assessed one month later, the treated plots showed on average a 94% reduction in the amount of shoot regrowth, a 65% reduction in the rhizome and tuber content of the soil and a 63% reduction in the viability of these tissues. The effects were attributed to the initial phytotoxic effects on the rhizome tissue and any residual fumigant in the soil preventing rhizome and shoot development.

Lawson (1984) made a study of the contribution of partial soil sterilants to weed control in soft fruit crops. He reported that the high cost of treatment has restricted their use in commercial crops to the control of nematodes and fungal pathogens. Experiments were done by injecting 1,3-D using a Rumpstadt Combiject to a depth of 20 cm and then lightly sealing the surface by rolling. They found that 1,3-D had little or no effect on the weed

seed population in the top 10 cm of the soil. However, the lack of effect could be because much of the gas escaped from the top 5 cm of soil. If samples had been taken to a depth of 20 cm, greater kill of the seeds may have been recorded. Control of organisms near the soil surface with fumigants is usually poor. The control achieved is a function of the chemical concentration × time of exposure. As the fumigant diffuses to the surface, the concentration decreases because there is usually more air space for diffusion and there is rapid loss from the soil, reducing the effectiveness of the chemical (Turner, Greathead & Welch, 1974).

A study on the effectiveness of using soil sterilants followed by either rolling or sheeting with polyethylene for weed control was done by Bond & White (1984). Trials with the soil fumigants dazomet, metham sodium and dichloropropene (92% a.i. Telone II at 450 l product ha^{-1}) showed that, if the plots were covered in sheeting, the results were equally good for all three chemicals and there were virtually no viable weed seeds in the top 10 cm of the soil. In plots treated with dichloropropene and covered by polyethylene sheeting, there was almost complete kill of weed seeds down to 25 cm depth. However, on rolled plots the seeds were still viable in the top 5 cm but were almost completely killed down to 25 cm depth. The dose of 1,3-D used in this experiment was almost double the rate used for PCN control but it still clearly shows that it is possible to kill weed seeds in the soil.

Soil fumigation with 1,3-D (Telone II) for broomrape (*Orobanche* spp.) control was tried by Jacobsohn *et al.* (1991). They found that 1,3-D was more toxic to seeds of *Orobanche crenata* which was completely controlled at a rate of 150 l ha^{-1} than to *Orobanche aegyptica* and *Orobanche muteli* which were only completely controlled using a rate of 450 l ha^{-1}. They found that there were not only fewer seedlings in the treated plots but they

were also slower growing.

2.0 Chapter 2.

The occurrence and distribution of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* in England and Wales

2.1 Introduction

Although PCN have been known about in the UK for nearly a hundred years, there has never been a statistically unbiased, systematic survey of their incidence in England and Wales and so a survey of potato growing land was undertaken. The survey collected soil samples from fields that had grown potatoes in the previous year and which were therefore part of the land currently in use for potato production. The survey was structured so that all growers who grew potatoes had an equal chance of being selected to be part of the survey irrespective of their location in England and Wales.

There are several procedures, using widely differing technologies, available for routine use in the identification of potato cyst nematode populations. These include the traditional approach of using morphological features (Stone, 1985; Golden, 1986), two dimensional electrophoretic analysis of protein profiles (Bakker & Gommers, 1982), analysis of protein patterns by isoelectric focusing (Fleming & Marks, 1982; Ibrahim & Rowe, 1995), ELISA (Fox & Atkinson, 1985; Schots *et al.*, 1989; Curtis *et al.*, 1998), polymerase chain reaction (PCR) based techniques (Ibrahim, Perry, Burrows & Hooper, 1994; Mulholland *et al.*, 1996; Bulman & Marshall, 1997; Ibrahim, Saad, Haydock & Al-Masri, 2000), randomly amplified polymorphic DNA (RAPD)-PCR markers (Burrows, Halford & Evans, 1996; Blok, Phillips & Harrower, 1997), restriction fragment length polymorphisms (RFLPs) and hybridisation techniques (Curran, Baillie & Webster, 1985; Burrows & Perry, 1988).

Each of these methods has problems for use in routine identification (Mulholland *et al.*, 1996) and none of them has been fully tested and validated against a range of field populations, with most of the work done only on known species from glasshouse cultures. The PCR technique offers the prospect of a simple, rapid and reliable diagnostic tool for

plant parasitic nematodes that will enable advisers to identify and quantify populations of PCN from field samples (Burrows & Perry, 1988; Stratford, Shields, Goldsbrough & Fleming, 1992; Ibrahim, Baldwin, Roberts & Hyman, 1997), as do ELISA based procedures (Evans *et al.*, 1995).

2.1.1 Aims

The main aims of the survey were to estimate the proportion of potato fields infested with PCN and to determine the relative abundance and distribution of the two species. The secondary aim was to compare results for the detection, quantification and identification of PCN populations using standard counting, PCR, IEF, ELISA and bait plant techniques on a sub-set of the survey samples.

2.2 Materials and methods

2.2.1 Selection of survey sites

A calculation was done in order to determine how many samples were required for the survey to accurately assess the proportion of fields that were infested with PCN (Appendix 2). An initial estimate of the proportion of infested fields was needed and this was based on a value obtained from the survey done by the PMB (Hancock, 1996). This calculation revealed that at least 96 sites would have to be sampled to accurately assess the proportion of fields that were infested. Previous studies (Jones & Pawelska, 1963; Guile, 1967, England, 1970) found that there were differences in the distribution of the species with *G. rostochiensis* especially common in East Anglia and south-east England while *G. pallida* was more common in Yorkshire and the East Midlands. Since the survey also aimed to assess the relative abundance and distribution of the two species, it was therefore decided than a larger number of samples would give a greater depth of information, especially in

those areas where fewer potatoes are grown. After consideration of the time available for collection and processing of the samples, it was decided that approximately 500 sites would be sampled.

The British Potato Council (BPC) list of registered growers was used to select growers for the survey. The BPC systematically selected growers who grew at least 2 ha of potatoes the previous year. The basis of the systematic selection process was to give every hectare of potato land an equal probability of selection that was not dependent on the size of the potato grower or the county in which it was grown. All of the growers were arranged in a table in rank order according to the area of potatoes that they grew. The growers who were invited to be part of the survey were systematically selected from this table.

An initial selection of 980 growers was contacted, requesting their participation in the survey. The letter sent to growers inviting them to be part of the survey is shown in Appendix 3. This produced 270 positive responses and a further 750 letters were sent out to increase the number of sites sampled. This produced another 214 replies and a total of 484 growers were included in the survey.

2.2.2 Sample collection

A soil sample was taken from one randomly selected field, by alphabetic selection of a field name from the list of fields, used by each grower for growing potatoes in the previous year. The cultivar of potatoes grown and a brief field history was recorded for each site sampled (Appendix 4). Samples were taken using a "cheese-corer" style auger with a half-cylindrical blade. Soil samples consisting of 50 cores (20 × 2.5 cm) were taken in a grid pattern to give approximately 2 kg of soil. A grid pattern was chosen as many of the sites

sampled had other crops at varying growth stages and sampling in this manner enabled samples to be taken along tramlines. This prevented damage to the crop, which would have resulted from sampling in a W-pattern. Evans, Niesten & Haydock (2000) found that a grid pattern was both consistent and accurate.

One soil sample was taken from sites 1-220. These were kept at Harper Adams University College for PCN extraction. Two soil samples were taken from sites 221-484, with the two samples consisting of equal numbers of cores taken from the same sampling points in the field. The first was put in a cotton bag and taken to Harper Adams University College for cyst extraction and processing in the IEF and PCR studies. The second was put in a plastic bag and sent to IACR-Rothamsted for a second Fenwick can extraction, bait plant testing and ELISA studies. A further 83 sub-samples, taken from those samples from batch 1-220 in which no cysts were found, were sent to Rothamsted for a second Fenwick can extraction and a bait plant test, giving a sub-set of 347 samples tested three times for the presence of cysts.

The area sampled was up to 4 ha for each site. The location of the area sampled was recorded using a Global Positioning System receiver. The number of samples collected from each county is shown in Table 2.1. The list of nearest towns and county from which all samples were taken is shown in Appendix 5. The number of Potato Marketing Board (PMB) registered producers in each county and potato plantings by PMB registered producers in each county is shown using 1996 data. This is because the selection of growers for inclusion in the survey was made in 1997 based upon data from 1996.

Table 2.1. *Number of samples collected in each county, number of Potato Marketing Board (PMB) registered producers in each county and potato plantings by PMB registered producers in each county in 1996 (Potato Statistics in Great Britain, 1993-1997)*

County	Number of samples collected	Hectares of potatoes grown	Number of PMB producers
England[a]			
Bedfordshire	3	494	76
Berkshire	0	80	13
Buckinghamshire	0	52	14
Cambridgeshire	48	9972	653
Cheshire	12	4511	359
Cornwall	16	3884	365
Cumbria	6	412	107
Derbyshire	7	748	96
Devon	6	1623	173
Dorset	1	121	15
Durham	3	962	58
East Sussex	2	165	20
East Yorkshire	13	5863	588
Essex	10	4759	210
Gloucestershire	3	981	91
Greater London	0	0	0
Greater Manchester	3	447	52
Hampshire	3	605	54
Hereford & Worcester	24	7613	398
Hertfordshire	4	264	24
Isle of Wight	0	374	13
Kent	16	3445	187
Lancashire	21	2827	311
Leicestershire	3	979	88
Lincolnshire	79	17659	1105
Merseyside	7	1015	112
Norfolk	45	12720	584
North Yorkshire	46	8965	887
Northamptonshire	0	347	31
Northumberland	1	1087	43
Nottinghamshire	9	4886	157
Oxfordshire	5	487	32
Shropshire	18	7839	282

Table 2.1. (continued)

County	Number of samples collected	Hectares of potatoes grown	Number of PMB producers
England[a]			
Somerset	9	1810	169
South Yorkshire	8	1098	118
Staffordshire	14	3616	191
Suffolk	9	5203	210
Surrey	0	98	12
Tyne and Wear	1	126	7
Warwickshire	6	1947	142
West Midlands	7	191	33
West Sussex	2	834	36
West Yorkshire	4	1037	96
Wiltshire	0	450	37
Wales			
Clwyd	1	139	44
Dyfed	5	1790	169
Gwent	1	382	63
Gwynedd	0	193	43
Mid Glamorgan	0	20	10
Powys	3	357	25
South Glamorgan	0	64	19
West Glamorgan	0	189	46
Total	484	125730	8080

[a]Due to county reorganisation, Avon has been included with Somerset, Cleveland with North Yorkshire and Humberside has been called East Yorkshire.

A correlation matrix was done between the number of samples taken in each county, number of Potato Marketing Board (PMB) registered producers in each county and potato plantings by PMB registered producers in each county in 1996 (Table 2.2). The results show that all three variables are strongly correlated. The number of samples collected from each county is strongly representative of the number of PMB registered producers in each county and the total area of potatoes grown in that county.

Table 2.2. *Correlation matrix between number of samples collected in each county, number of Potato Marketing Board (PMB) registered producers in each county and potato plantings by PMB registered producers in each county in 1996 (Potato Statistics in Great Britain, 1993-1997)*

Correlation	Correlation Value (r)
Number of samples collected * Number of PMB producers	0.95
Number of samples collected * Hectares of potatoes grown	0.94
Number of PMB producers * Hectares of potatoes grown	0.94

2.2.3 Sample processing

2.2.3.1 Standard methods of cyst detection and estimation of cyst contents

For sites 1-220, soil samples were air-dried and sieved through a 4 mm aperture sieve. Each soil sample was thoroughly mixed by stirring with a spoon, and a 200g sub-sample was taken from each for cyst extraction using a Fenwick can (Fenwick, 1940). The numbers of *Globodera* cysts and their cyst contents were estimated by standard methods (Southey, 1970). The numbers of PCN cysts present in each extract were counted under a

stereomicroscope and, where possible, 50 cysts (all cysts if there were less than 50) were removed for egg counts. The cysts were soaked in water overnight before crushing to release the eggs. From the resulting suspensions the eggs in 1 ml aliquots were counted. A population density in eggs g^{-1} of dried field soil was calculated for each sample in which PCN was found. Samples that contained cysts other than *Globodera* species were noted but their species was not determined.

For sites 221-484, the first sample was processed as described above. The second sample was used for a separate Fenwick can extraction and a bait plant test (2.2.3.2). The numbers of *Globodera* cysts and their cyst contents were estimated by standard methods and other cyst nematode species were identified by morphological methods (Appendix 7).

2.2.3.2 Bait plant tests

For the bait plant tests, a 25% admixture of peat-based compost was added to the field soils in order to prevent compaction in the pots. 400 g of these modified soils (347 samples in all) were added to 10 cm diameter plastic pots in which potato plants of the cultivar Désirée were grown for 12 weeks before extracting by Fenwick can any new cysts that had formed.

2.2.4 Species identification

When sufficient cysts were available, the species present in samples were identified by isoelectric focusing (IEF) (Fleming & Marks, 1983) using between 25 and 50 cysts (but sometimes, by necessity, fewer) and by using the polymerase chain reaction (PCR) (Ibrahim *et al.*, 2000). Species identifications in samples that contained insufficient cysts for identification by IEF were made by PCR.

2.2.4.1 DNA Extraction

DNA was extracted as described by Ibrahim *et al.* (1997). The suspensions of crushed cysts remaining from the egg counts were used as a source of DNA template for the PCR reactions. The remaining suspension was centrifuged at 500 rpm for 5 minutes, after which the pellet was re-suspended in water in a 1.5 ml microcentrifuge tube before centrifuging at 12 000 rpm for 5 minutes. The excess water was removed and 20 µl of lysis buffer (10 mM Tris (pH 8.0), 1 mM EDTA, 1% Nonidet P-40, 100 µg/ml proteinase K) were added. The pellet was homogenised with a micro-homogeniser (Biomedix, UK) and the extract incubated at 95^0C for 5 minutes. The crude DNA extracts prepared in this way were suitable for PCR amplification without further treatment.

2.2.4.2 PCR Amplification

Two different sets of PCR primers were used in this study. In the set described by Bulman & Marshall (1997), the G. *rostochiensis* primer (5' -AGCGCAGACATGCCGCAA-3') (PITSr3) binds to the ITS3 region and the *G. pallida* primer (5'-ACAACAGCAATCGTCGAG-3') (PITSP4) binds to the ITS4 region. They were used in combination with a universal primer (5'-GGAAGTAAAAGTCGTAACAAGG-3') that binds to the ITS5 region (White, Burns, Lee & Taylor, 1990). The second set of primers (Mulholland *et al.*, 1996) are designed to bind to the ITS1 region and the 5.8S rRNA gene. The *G. rostochiensis*-specific primer was 5'-TGTTGTACGTGCCGTACCTT-3', the *G. pallida*-specific primer 5'-GGTGACTCGACGATTGCTGT-3', and the universal primer was 5'-GCAGTTGGCTAGCGATCTTC-3'. Each sample was run at least three times with each set of primers.

2.2.4.3 Agarose gel electrophoresis

PCR products were separated on 1.2% agarose gel buffered in 1 × TEB, which contained 0.02 μg/ ml ethidium bromide. DNA was visualised under UV light and records made with a digital camera attached to a Gel Doc-1000 box (Bio-Rad Ltd).

2.2.4.4 Protein Extraction and IEF gel electrophoresis

Where sufficient cysts were available in a sample, 50 were taken and soaked in distilled water overnight. Where fewer cysts were available, as many as possible were taken (usually between 25 and 50 but sometimes less). The cysts of each sample were transferred to100 μl of 1% glycerol, kept on ice and homogenised using a Biomedix homogeniser, and then centrifuged at 14 000 rpm for 10 minutes at 40C. Proteins in the samples were separated using an LKB Multiphor isoelectric focusing (IEF) gel system with ampholine pH range of 4 to 6 (Ibrahim & Rowe, 1995).

Cathode and anode electrode strips were soaked in 1.0 M NaOH and 1.0 M H_3PO_4 respectively. The gel was pre-focused for 30 min with 800 V at 40C, followed by electrophoresis for 2 h at 2000 V. Protein bands on the gels were stained with 0.5% Coomassie Brilliant blue R250 in 25% ethanol and 10% acetic acid at 60^0C for 30 min. Coomassie-stained gels were subsequently destained with several changes of 50% ethanol, 7% acetic acid solution. Since silver staining techniques are up to 100-fold more sensitive than using Coomassie blue, gels that stained only faintly with Coomassie blue were re-stained with silver staining, using the procedure described by Oakley, Kirsch & Morris (1980). Samples of standard, known populations both *G. pallida* and *G. rostochiensis* cysts were included on the gel to help in identification of the species specific bands that occur

with isoelectric points at pH 5.7 and 5.9 respectively. Each sample was run at least twice to confirm the reproducibility of the banding patterns.

2.2.4.5 Enzyme-linked immunosorbent assay (ELISA)

Twelve cysts were taken from each population. Where insufficient cysts were available from the first counts of cysts in samples, further cysts were extracted from any remaining soil. Where there were still insufficient, cysts were taken from the bait plant test. If fewer than 12 cysts were available, the ELISA identifications were not performed. Each cyst from each sample was homogenised individually, in 10 µl of PBS, in 96-well culture plates using a micropipette tip as a homogeniser. The wells and pipette tips were washed with 200 µl of PBS, and the resulting solution was divided between two wells of an ELISA microtitre plate, each of which was then serially diluted (×2) three times. Plates were left overnight at room temperature and then washed three times with PBST (0.05% v/v Tween, in PBS) and probed with each of the two PCN species-specific monoclonal antibodies in PBSTM (0.05% v/v Tween, 5% w/v dried skimmed milk in PBS). The secondary antibody used was rabbit anti-rat IgG conjugated with horseradish peroxidase. Following detection with substrate, the optical densities (OD) of the samples were read on a Titertek ELISA plate reader. Any cyst producing an OD of less than 50 was deemed unclassifiable; when ODs were between 50 and 100, a difference of greater than 20 OD units was required between antibodies before classification as one species or the other; when ODs were over 100, a difference of greater than 30 OD units was required between antibodies before classification as one species or the other. The OD readings of the individual cysts varied largely as a consequence of their differing egg contents, and the average OD per unequivocally classified cyst was calculated for each species in each sample. The ODs of these unequivocally classified cysts were also summed for each species in each sample.

2.2.5 Statistical analysis and data handling

To determine statistical differences between nematode sampling techniques for the generated binary response data (e.g. nematodes/cysts present or absent), data were analysed by "Statistica" (Stratsoft Inc. 1999) using a generalised linear model, assuming Poisson errors and a log-link function. This type of analysis is appropriate when there are no continuous explanatory variables associated with binary responses and the explanatory variables are essentially factors (Crawley 1993). Statistical calculations are based on maximum likelihood estimation and produce the Wald statistic, which is then tested against the chi-square (X^2) distribution for significance. Chi-square tests for association were done to determine if there were relationships between factors.

In addition, some of the data from the experiment were analysed using Genstat^{TM} 5, Release 4.1, (Lawes Agricultural Trust, IACR-Rothamsted, UK). All data were checked to establish whether they had normal distributions. A general analysis of variance was done where possible on untransformed data but the data were transformed when necessary.

2.3 Results

2.3.1 Cyst detection

The results of standard counts and bait plant tests are presented in Table 2.3. The first Fenwick can extraction and cyst counts done at Harper Adams revealed that 269 out of 484 (56%) of the field samples tested contained PCN cysts, but that cysts extracted from only 237 out of 484 (49%) of the samples contained live eggs. The results for the counts on the sub-set of 347 samples done at both Harper Adams and Rothamsted were similar to each other with 38% and 36% respectively of samples containing cysts. Statistically significant

differences in the proportion of cysts containing eggs were observed at the two laboratories; 26% of cysts were found to contain eggs at Rothamsted whilst eggs were found in 34% of cysts at Harper Adams. The bait plant tests revealed cysts in 148 samples compared to the 126 that were positive from the first field soil count. A combination of the bait plant tests and the second Fenwick can extraction made directly on field soils increased the overall detection of PCN in the field samples to 64% from the 56% found in the first direct counts (Table 2.3).

Table 2.3. *Detection of potato cyst nematodes,* Globodera *spp., by direct extraction using Fenwick can of field soil or field soil in which bait plants had been grown. Numbers in parenthesis are 95% confidence limits.*

Method	Number of samples tested	Number of samples containing cysts	% samples containing cysts	Number of samples containing eggs	% samples containing eggs
Fenwick HAUC[a]	484	269	56	237	49
Fenwick HAUC[b]	347	132	38 (± 5.1)	119	34 (± 5.0)
Fenwick IACR[b]	347	126	36 (± 5.1)	90	26 (± 4.6)
Bait test	347	148	43 (± 5.2)	128	37 (± 5.1)
Wald X^2 (2DF)			1.90		6.96*
All methods	484	311	64	263	54

*$P < 0.05$

[a] total samples tested at Harper Adams University College only
[b] sub-set of the total samples tested at both HAUC and IACR-Rothamsted

Key findings

- PCN cysts were detected in 64% of sites sampled
- the initial extraction detected cysts in only 56% samples
- 8% of infestations detected were cryptic and were only detected after a second extraction and bait plant test

2.3.2 Population densities

The population densities were measured for each sample where an infestation was detected. The results were expressed in eggs g^{-1} soil and are shown in Table 2.4. The results shown are for the population counts done after the standard Fenwick can extraction at Harper Adams. However, population counts for infestations detected by the standard extraction at Rothamsted but not Harper Adams were also included. Results for population densities obtained after bait plant tests were not included as they do not represent field population densities.

Of the categories of infestation used, the most common population density was <10 eggs g^{-1} soil (62.1% of infested sites). Over 94% of infested sites had a population density of less than 60 eggs g^{-1} soil. The mean population density was 15 eggs g^{-1} soil and the median population density was 7 eggs g^{-1} soil.

Key findings

- over 62% of population densities were less than 10 eggs g^{-1} soil
- over 94% of infested sites had a population density of less than 60 eggs g^{-1} soil
- the mean population density was 15 eggs g^{-1} soil

Table 2.4. *Number and percentage of sites containing different population densities of potato cyst nematodes (eggs g^{-1} soil)*

Population density (eggs g^{-1} soil)	Number of sites	% of infested sites
<10	149	62.1
10-20	41	17.1
20-30	15	6.3
30-40	8	3.3
40-50	7	2.9
50-60	6	2.5
60-70	2	0.8
70-80	3	1.3
80-90	1	0.4
90-100	3	1.3
>100	5	2.1

2.3.3 Species identification

2.3.3.1 Species determination by all methods

The species of PCN were determined for all of the populations where sufficient material was available. 254 population identifications were made by PCR and 83 by IEF. An example of the IEF gel used for the identification of the populations is shown in Plate 2.1. The complete results for the species determination of samples by PCR, IEF and ELISA are shown in Appendix 6. In this gel, no band was visible for sample 11 but samples 4 and 20 contained bands indicating that they were *G. rostochiensis* and all other samples were *G. pallida*. The species determination results for the samples found to contain cysts are in Table 2.5: 174 samples contained *G. pallida* (Pa), 21 contained *G. rostochiensis* (Ro), and 66 contained both species. The number of samples containing cysts is expressed as a

percentage of the total number of sites sampled (484 sites) and also expressed as a percentage of the samples for which a species determination was made (261 samples).

Table 2.5. *Number and percentages of samples found to contain* Globodera pallida *(Pa),* G. rostochiensis *(Ro), or both species (Pa + Ro)*

	Species present		
	Pa	Ro	Pa + Ro
Number of samples	174	21	66
% of total samples	36	4	14
% of samples containing cysts	67	8	25

Other species of cyst nematodes such as *Heterodera* spp. and fungal fruiting bodies may occur in PCN infested samples and both can be confused in appearance with PCN cysts. These may inadvertently be included along with PCN cysts in samples for species identification and any species identification method must therefore be specific for *Globodera* species only. Extracts of other cyst nematodes (*Heterodera* spp.) and a range of unidentified fungal fruiting bodies found in the field samples were also run with the two sets of PCR primers and no amplification products were observed indicating that the primers were specific to *Globodera* species only.

Key findings

- 67% of populations were *G. pallida*
- 8% of populations were *G. rostochiensis*
- 25% of populations contained both *G. pallida* and *G. rostochiensis*

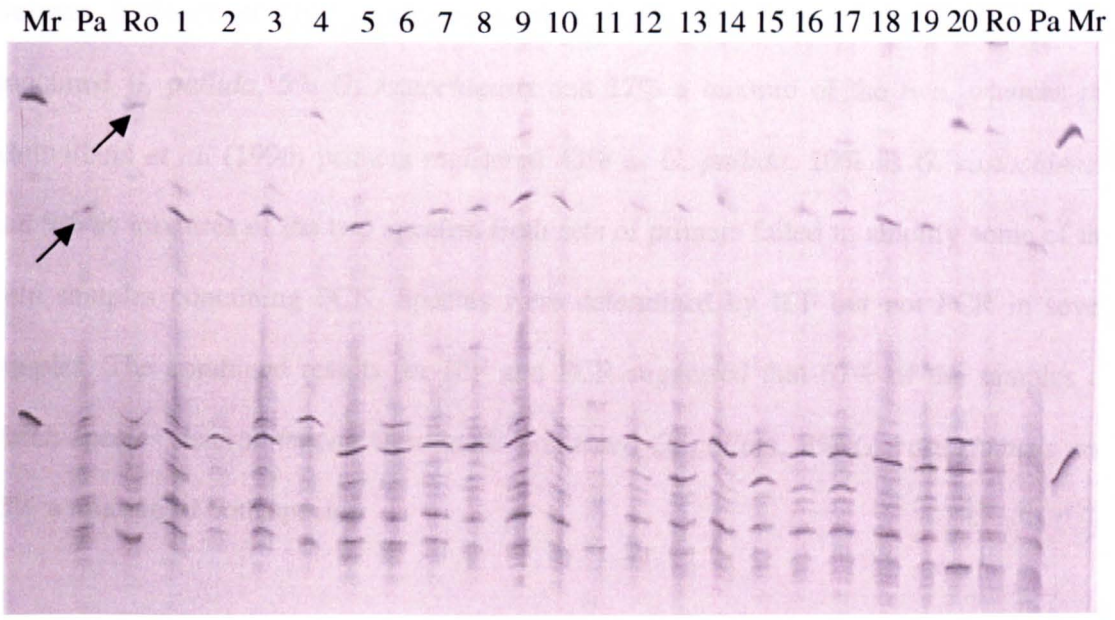

Plate 2.1. The identification of *G. rostochiensis* and *G. pallida* species in populations collected during the survey using iso-electric focusing on a polyacrylamide gel (Mr-Marker, Pa –*G. pallida*, Ro -*G. rostochiensis*). The two arrows point to the species-specific bands used for the identification of each species.

2.3.3.2 Comparisons between species identification methods

The results for the identification of PCN in all samples by all of the different methods are summarised in Table 2.6. Both sets of PCR primers distinguished the two species of PCN and both identified some populations as mixtures. The combined results from the two sets of primers suggested that 64% of the samples for which a PCR result was obtained contained *G. pallida*, 7% *G. rostochiensis* and 25% a mixture of the two species. The Bulman & Marshall (1997) primers alone revealed that 53% of the samples tested contained *G. pallida*, 5% *G. rostochiensis* and 17% a mixture of the two, whereas the Mulholland *et al.* (1996) primers registered 43% as *G. pallida*, 10% as *G. rostochiensis* and 9% as mixtures of the two species. Both sets of primers failed to amplify some of the field samples containing PCN. Species were determined by IEF but not PCR in seven samples. The combined results for IEF and PCR suggested that 67% of the samples of which species determinations were made contained *G. pallida*, 8% *G. rostochiensis* and 25% a mixture of both species.

The Bulman & Marshall (1997) primers generated products in 75% of the 269 samples tested, and the Mulholland *et al.* (1996) primers in 62%. The two sets of primers did not always generate identification products for the same populations with, occasionally, one population identified by one set of primers but not the other set. The reason why both sets of primers failed to give a species identification is unclear and requires further investigation. However, in none of the samples tested, was one species detected by one set of primers and the other species detected by the second set of primers. The lengths of the amplification products are 434 bp for the *G. rostochiensis* fragment and 265 bp for the *G. pallida* fragment using Bulman & Marshall (1997) primers, and 391 bp and 238 bp, respectively, using Mulholland *et al.* (1996) primers.

Table 2.6. *Identification of the potato cyst nematodes* Globodera rostochiensis *(Ro) and* G. pallida *(Pa) using different PCR primers, IEF and ELISA. Numbers in parenthesis are 95% confidence limits.*

Method	Number of samples tested	Samples containing Pa (%)	Samples containing Ro (%)	Samples containing Pa + Ro (%)	Samples with no species detected (%)
Bulman & Marshall	269	53 (±6.0)	5 (±2.7)	17 (±4.5)	25 (±5.2)
Mulholland *et al.*	269	43 (±6.0)	10 (±4.0)	9 (±3.4)	38 (±5.9)
Both PCR	269	64 (±6.0)	7 (±3.1)	25 (±5.2)	4 (±2.3)
IEF	83	83 (±8.2)	12 (±7.2)	5 (±3.7)	0
ELISA	93	19 (±8.2)	19 (±8.2)	61 (±10.0)	0
Wald X^2		42.33*** (4DF)	16.42** (4DF)	91.31*** (4DF)	50.99*** (2DF)

$^{**}P < 0.01$, $^{***}P < 0.001$

IEF and ELISA tests could only be used on 83 and 93 samples respectively, because insufficient cysts were available in the remainder of samples. Protein banding patterns of all the populations examined were highly reproducible. Species determination by both IEF and PCR was made for 76 populations. The same results were obtained for 64 of the 76 populations but, for 12 populations, PCR detected two species where IEF found only one species. ELISA was performed on 93 samples but it was impossible to decide whether a cyst was one species or the other in many cases. From the cysts that were unequivocally identified, 61% of the samples appeared to be mixtures of the two species, 19% *G. pallida*, and 19% *G. rostochiensis*. However, there were many cysts that could not be

unequivocally ascribed to either species and only about one in three populations appeared to be either *G. pallida* or *G. rostochiensis.*

Statistical analysis of the results found that there were significant differences between the identification methods in the percentage of samples found to contain *G. pallida, G. rostochiensis* and in the samples containing both *G. pallida* and *G. rostochiensis.* However, the number of samples tested by each method varied from 93 to 269 samples and so the differences between methods could be as a result of the tests being done on different subsets of samples.

Thirty samples from the survey were tested by all methods and the results are shown in Table 2.7. Statistical analysis of the results found that there were significant differences between methods in the percentage of samples containing only *G. pallida* and in the samples containing both *G. pallida* and *G. rostochiensis.* However, this was no statistical significance between methods in the percentage of samples *G. rostochiensis* or in the number of samples with no species detected by both sets of PCR primer. The Mulholland *et al.* (1996) primers, IEF and ELISA were in almost full agreement for the identification of *G. rostochiensis* (17%, 20% and 17% respectively). The two sets of PCR primers taken together were in moderate agreement with the results from IEF in terms of the percentage of samples tested that contained only *G. pallida* but disagreed on the percentage of samples tested that contained only *G. rostochiensis.* The differences between methods were in the percentages of populations that were determined to be mixtures of the two species. No populations were determined as one species by one method but the other species by a different method. IEF did not identify any of this subset of 30 populations as a mixture of the two species. However, both PCR and ELISA registered populations as mixtures of the

two species, but disagreed greatly on the number of mixed populations (20% and 67% respectively). The results for the species identification in Appendix 6 show that there were a large number of populations that were found only to contain *G. pallida* by PCR and IEF but were found to contain both species by ELISA although the reasons for this are unknown.

Key finding

- all methods were able to distinguish the two species of PCN

Table 2.7. *Identification of potato cyst nematodes* Globodera rostochiensis *(Ro) and* G. pallida *(Pa) in 30 samples using different PCR primers, IEF and ELISA. Numbers in parenthesis are 95% confidence limits.*

Method	Number of samples tested	Samples containing Pa (%)	Samples containing Ro (%)	Samples containing Pa + Ro (%)	Samples with no species detected (%)
Bulman & Marshall	30	47 (±18.9)	3 (±7.0)	10(±11.0)	40(±18.7)
Mulholland *et al.*	30	43 (±18.8)	17(±14.1)	7(±10.0)	33(±17.9)
Both PCR	30	73 (±16.8)	7(±9.4)	20(±15.0)	0
IEF	30	80 (±15.2)	20(±15.1)	0	0
ELISA	30	17 (±14.1)	17(±14.3)	67(±17.9)	0
Wald X^2		13.23* (4DF)	4.18 (4DF)	20.20** (3DF)	0.18 (1DF)

*$P < 0.05$, **$P < 0.01$

2.3.4 Species distribution

The areas of England and Wales in which more than 1% of the land was used to grow potatoes in 1998 are shown in Fig. 2.1. Fig. 2.2 shows the sites where PCN were found and Fig. 2.3 shows the sites where no PCN were found. Figs 2.2 and 2.3 show how the sites sampled were located in the main potato growing regions seen in Fig. 2.1. In addition, Fig. 2.3 shows that sites found not to contain PCN are evenly distributed across the country. In contrast, Fig. 2.2 shows that the sites that did contain PCN were more likely to be concentrated in the counties that are more intensive potato growing areas.

Fig. 2.4 shows the sites where *G. pallida* was detected and Fig. 2.5 shows the sites where *G. rostochiensis* was detected. Sites that contained both species are shown on both Figs 2.4 and 2.5. *Globodera pallida* is concentrated in the eastern counties of Lincolnshire, Yorkshire and Cambridgeshire where many potatoes are grown. In these regions, few sites were found not to contain cysts (compare Figs 2.2 and 2.3). *Globodera rostochiensis* was present in eastern and western counties almost equally, frequently in the same locations as *G. pallida* (compare Figs 2.4 and 2.5). Fig. 2.3 shows that most sites in southern England were free from PCN, as were parts of Shropshire and Hereford and Worcester.

Key findings

- both species were found more often in the main potato growing areas
- *G. pallida* was found more often in the eastern counties of Lincolnshire, Yorkshire and Cambridgeshire
- *G. rostochiensis* was present in eastern and western counties almost equally
- southern England, Shropshire and Hereford & Worcester were mainly free from PCN

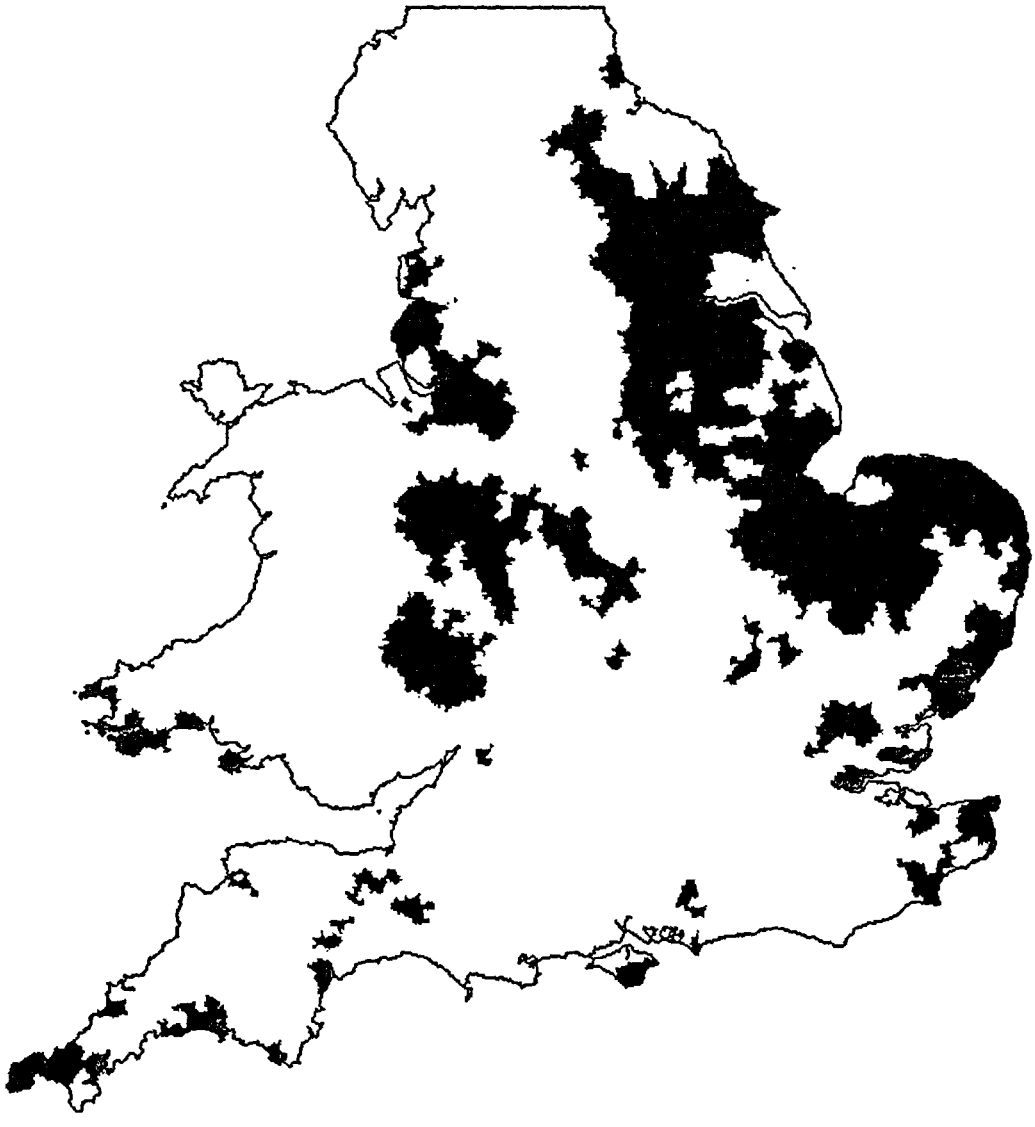

Fig. 2.1. Areas of England and Wales with more than 1% of land used for potato production (Agricultural Census Statistics for the United Kingdom, 1998).

Fig. 2.2. Sites where potato cyst nematodes were found.

Fig. 2.3. Sites where no potato cyst nematodes were found.

Fig. 2.4. Sites which contained *Globodera pallida* cysts.

Fig. 2.5. Sites which contained *Globodera rostochiensis* cysts.

2.3.5 Previous potato cultivars grown

When possible, the previous cultivars grown on each of the sites sampled were recorded (Table 2.8). Many of the sites sampled were cropped with more than one cultivar giving a total of 582 records. The results showed that cultivars that had resistance to *G. rostochiensis* Ro1 were grown 249 out of 582 times (43%) and those that had resistance to *G. pallida* Pa2/3 were grown 35 times out of 582 records (6%).

Key findings

- cultivars with resistance to *G. rostochiensis* Ro1 were grown 249 out of 582 times (43% of times)
- cultivars with resistance to *G. pallida* Pa2/3 were grown 35 times out of 582 records (6% of times).

Table 2.8. *Numbers and percentages of the previous cultivars grown on the sites sampled that are resistant (r) or susceptible (s) to Globodera rostochiensis Ro1 (Ro) or G. pallida Pa2/3 (Pa)*

Cultivar	Ro resistance	Pa resistance	Number grown	Number of Ro resistant cultivar grown	Number of Pa resistant cultivar grown
Nadine	r	r	20	20	20
Rocket	r	r	9	9	9
Santé	r	r	5	5	5
Swift	r	r	1	1	1
Maris Piper	r	s	99	99	-
Cara	r	s	36	36	-
Picasso	r	s	12	12	-
Fianna	r	s	11	11	-
Morene	r	s	11	11	-
Pentland Javelin	r	s	6	6	-
Saturna	r	s	6	6	-
Stemster	r	s	5	5	-
Anna	r	s	4	4	-
Minerva	r	s	4	4	-
Dundrod	r	s	3	3	-
Saxon	r	s	3	3	-
Ausonia	r	s	2	2	-
Lady Rosetta	r	s	2	2	-
Adora	r	s	1	1	-
Ambo	r	s	1	1	-
Balmoral	r	s	1	1	-
Caesar	r	s	1	1	-
Cultra	r	s	1	1	-
Kingston	r	s	1	1	-
Nicola	r	s	1	1	-
Penta	r	s	1	1	-
Premiere	r	s	1	1	-
Symfonia	r	s	1	1	-
Estima	s	s	64	-	-
Maris Bard	s	s	39	-	-
Marfona	s	s	36	-	-
Wilja	s	s	35	-	-
Desiree	s	s	34	-	-
Pentland Dell	s	s	26	-	-

Table 2.8. (continued)

Cultivar	Ro resistance	Pa resistance	Number grown	Number of Ro resistant cultivar grown	Number of Pa resistant cultivar grown
Romano	s	s	17	-	-
Record	s	s	15	-	-
Pentland Squire	s	s	9	-	-
R. Burbank	s	s	9	-	-
King Edward	s	s	5	-	-
Hermes	s	s	4	-	-
Maris Peer	s	s	4	-	-
Bintje	s	s	2	-	-
Brodick	s	s	2	-	-
Carlingford	s	s	2	-	-
Charlotte	s	s	2	-	-
Colmo	s	s	2	-	-
Kondor	s	s	2	-	-
Ostara	s	s	2	-	-
Pentland Hawk	s	s	2	-	-
Shepody	s	s	2	-	-
Alcmaria	s	s	1	-	-
Aminca	s	s	1	-	-
Charger	s	s	1	-	-
Erntestolz	s	s	1	-	-
Fambo	s	s	1	-	-
Fresco	s	s	1	-	-
Hbarna	s	s	1	-	-
Home Guard	s	s	1	-	-
Hurtha	s	s	1	-	-
Merlin	s	s	1	-	-
Pentland Crown	s	s	1	-	-
Pentland Marble	s	s	1	-	-
Prince	s	s	1	-	-
Riband	s	s	1	-	-
Sangre	s	s	1	-	-
Scotch Premier	s	s	1	-	-
Thambo	s	s	1	-	-
Vanessa	s	s	1	-	-
Total			582	249	35
% total				43	6

2.3.6 Rotation length

The rotation length was determined for 407 of the sites sampled (Table 2.9). The most common rotation length was 1 in 5 (29% of sites). 51% of sites had a rotation length of 1 in 5 or less and 80% of sites had a rotation length of 1 in 7 or less.

Table 2.9. *The rotation length of the sites sampled*

Rotation length (years)	Number of sites	% of sites sampled
1	4	1
2	6	1
3	18	4
4	60	15
5	119	29
6	84	21
7	36	9
8	15	4
9	7	2
10	23	6
11-20	12	3
>20	23	6

Key findings

- the most common rotation length was 1 in 5 (29% of sites)
- 51% of sites had a rotation length of 1 in 5 or less

2.3.7 Incidence of PCN and cultivar grown

A chi-square test for association was done to determine whether there was a relationship between the incidence of PCN and the resistance characteristics of the cultivar. The results show that there was a significant relationship ($P = 0.006$) between the incidence of PCN and the resistance characteristics of the cultivar grown (Table 2.10). An analysis was done to determine which values in the test contributed most to the value of X^2 (Neave & Worthington, 1988). Calculations indicated that cultivars resistant to *G. rostochiensis* would be expected to be grown 81 times when PCN were absent and 59 times when PCN were present if there was no relationship between incidence of PCN and cultivar grown. The results show that cultivars resistant to *G. rostochiensis* were grown more often (96 occasions) when PCN were present, and that they were grown less frequently (44 occasions) when PCN were absent indicating there is a significant relationship between the incidence of PCN and the resistance characteristics of the cultivar grown. It was also found that in the absence of PCN, more non-resistant cultivars (100 sites) were grown than would be expected (86 sites) if there was no relationship between incidence of PCN and cultivar grown.

Key findings

- cultivars resistant to *G. rostochiensis* were grown more often when PCN were present
- cultivars resistant to *G. rostochiensis* were grown less frequently when PCN were absent
- in the absence of PCN, more non-resistant cultivars were grown than would be expected

Table 2.10. *The effect of the use of resistant and non-resistant cultivars on the incidence of potato cyst nematodes*

	Incidence of PCN (no. of sites)		
Cultivar (no. of times grown)	Present	Absent	Total
Non-resistant	105	100	205
Ro resistant	96	44	140
Pa + Ro resistant	10	9	19
Total	211	153	364

X^2=10.19 (2df)

P = 0.006

2.3.8 Species detected and cultivars grown

Another chi-square test for association was done to determine whether there was a relationship between the species of PCN present and the resistance characteristics of the cultivars grown on each site. The results (Table 2.11) show that there was a significant relationship ($P < 0.001$) between the species of PCN present and the use of resistant and non-resistant cultivars. Calculations of the expected values indicate that there would be 78 non-resistant cultivars grown and 53 cultivars resistant to *G. rostochiensis* grown where *G. pallida* was detected if there was no relationship between incidence of PCN and cultivar grown. The observed values show that there were 56 non-resistant cultivars grown and 83 resistant cultivars grown with resistance to *G. rostochiensis*. This indicates that in the presence of *G. pallida*, significantly fewer numbers of non-resistant cultivars were grown and significantly higher numbers of resistant cultivars were grown. It was also found that

fewer cultivars resistant to G. *rostochiensis* were grown on sites free from PCN (44 sites) compared with what the expected number (59 sites) if there was no relationship between incidence of PCN and cultivar grown. This indicates that there was a significant relationship between the species of PCN present and the use of resistant and non-resistant cultivars.

Key findings

- there was a significant relationship between the species of PCN present and the use of resistant and non-resistant cultivars

Table 2.11. *The effect of the use of resistant and non-resistant cultivars on the species of potato cyst nematode present*

	Species present (no. of sites)				
Cultivar (no. of times grown)	Pa	Ro	Pa + Ro	None	Total
Non-resistant	56	14	35	100	205
Ro resistant	74	4	18	44	140
Pa resistant	9	0	1	9	19
Total	139	18	54	153	364

X^2=24.56 (3df)

$P < 0.001$

2.3.9 Species detected and population density

The relationship between the species of PCN present and the population density for each site was investigated and the results are shown in Table 2.12. The results show that sites that contained only *G. pallida* had significantly higher population densities than sites that contained only *G. rostochiensis* ($P < 0.001$). Although sites that contained a mixture of both species had higher population densities than those containing only *G. rostochiensis* and lower than those that contained *G. pallida,* these differences were not statistically significant.

Key finding

- sites that contained only *G. pallida* had significantly higher population densities than sites that contained only *G. rostochiensis*

Table 2.12. *The effect of the species of potato cyst nematode present on the population density (eggs g^{-1} soil) (back transformed means in parenthesis)*

	Species present		
	Pa	Ro	Pa + Ro
Log_e (eggs g^{-1} soil)	2.318 (10)	1.616 (5)	1.868 (6)

SED	Significance (P =)	df	CV%
0.3245 max-min	0.020	172	55.2

2.3.10 Cultivar and population density

The relationship between the effect of the use of resistant and non-resistant cultivars on the population density was also investigated (Table 2.13). The results show that there were lower population densities when cultivars with resistance to *G. pallida* were grown but these differences were not statistically significant.

Table 2.13. *The effect of resistant and non-resistant cultivars on the population density of infested sites (eggs g^{-1} soil) (back transformed means in parenthesis)*

	Cultivar		
	Pa resistant	Ro resistant	Non-resistant
Log_e (eggs g^{-1} soil)	1.365 (4)	2.064 (8)	1.907 (7)

SED	Significance (P =)	df	CV%
0.4308 max-min	0.246	189	66.5

2.4 Discussion

Globodera cysts were found in 64% of samples tested by standard detection methods and by bait plant tests. This figure is significantly higher than the figure of 42.1% from the subjective survey made by the PMB in 1992 (Hancock, 1996). The results from this survey were not based upon the analysis of samples, but on asking growers to estimate the levels of infestations on their fields (Storey, personal communication). This means that low population densities with few visible symptoms may have gone unreported. It has been

estimated that the incidence PCN in ware-growing areas ranged from 22% in Scotland (Evans, Harling & Dubickas, 1998), through 26% in Northern Ireland (Turner, 1996*b*) to 67% in England and Wales (Hancock, 1996). The estimate of 67% by Hancock (1996) was based on a sub-set of soil samples sent to ADAS for advisory reasons in 1994 and 1995. The information was gathered for other purposes and was not part of a systematic survey.

As potato production has become more specialised, with a reduction in the number of growers, production has been concentrated on a smaller area of land leading to shorter rotations that have encouraged build-up of PCN infestations (Anon, 1997; Haydock & Evans, 1998). This has been confirmed by the survey, which found that just over 50% of the sites sampled had a rotation length of 1 in 5 or lower (Table 2.9). Since decline rates for *G. pallida* are lower than that for *G. rostochiensis* (Evans, 1993), this shortening of rotations will reduce population decline for many growers.

The population densities of the infestations found were mainly low with about 62% below 10 eggs g^{-1} soil and only 6% above 60 eggs g^{-1} soil. These results are similar to those obtained by Hancock (1988). Of the infestations that were found, about 65% were classified (using the ADAS category scheme) as low (below 10 eggs g^{-1} soil), 26% as moderate (between 10 and 60 eggs g^{-1} soil) and 10% as high (greater than 60 eggs g^{-1} soil). The results indicate that most growers do not have significantly high populations.

Species identification results from samples processed by ADAS found 5% of populations to be predominantly *G. rostochiensis*, 54% predominantly *G. pallida* and 41% to be mixed populations (Hancock, 1996). This is similar to the present results, in which 8% of populations were *G. rostochiensis*, 67% were *G. pallida* and 25% contained both species.

Since *G. pallida* was found in 92% of samples compared to only 33% of samples for *G. rostochiensis*, *G. pallida* is clearly the commoner species in England and Wales. Earlier surveys (Dixon *et al.*, 1968; Brown, 1970) estimated a lower incidence for *G. pallida*, so this suggests that growing cultivars resistant to *G. rostochiensis* has selected *G. pallida* in field populations.

There are currently no cultivars with full resistance to *G. pallida* but there are some with partial resistance, which will reduce nematode multiplication but not prevent it. There is a risk that over-use of partially resistant cultivars may lead to selection of virulent populations (Haydock & Evans, 1998), but it is difficult for growers to deploy even this degree of resistance against *G. pallida* when the greatest market demand is for cultivars which have either no resistance at all or *G. rostochiensis* resistance only. Evans & Haydock (2000) commented that market appeal more than resistance has contributed to the popularity of Maris Piper which has resistance to *G. rostochiensis*. The survey results show that cultivars with partial resistance to *G. pallida* represented only 6% of the total number of plantings while those with resistance to *G. rostochiensis* represented 43% (Table 2.8). Calculations based on potato plantings by Potato Marketing Board registered producers showed similar results. In 1996, 7% of the total area of cultivars planted had partial resistance to *G. pallida* and 45% had resistance to *G. rostochiensis* (Anon, 1997).

The results showed that there was a significant relationship between the incidence of PCN and the resistance characteristics of the cultivar grown, with cultivars resistant to *G. rostochiensis* being grown more often when PCN were present, and less frequently when PCN were absent. The fact that resistant cultivars were grown more often in the presence of PCN suggest that growers were aware of their PCN problems and were trying to take

steps towards managing their problem by their choice of cultivar. The fact that fewer resistant cultivars were grown in the absence of PCN and more non-resistant cultivars were grown suggests that growers were aware that they had no PCN problem and their choice of cultivar was based more on the most suitable cultivar for the market place rather than on the need to control PCN.

There was a significant relationship between the species of PCN present and the use of resistant and non-resistant cultivars. There was a small number of cultivars grown with resistance to *G. pallida* and the numbers in each category did not vary from what would be expected if there was no relationship between the species present and the resistance of the cultivar. The results show that in the presence of *G. pallida*, significantly fewer numbers of non-resistant cultivars were grown and significantly higher numbers of cultivars resistant to *G. rostochiensis* were grown. This again suggests that growers were aware of PCN problems and were deliberately choosing resistant cultivars. However, the growers should have been choosing cultivars with resistance to *G. pallida* since on these sites, it was *G. pallida* that was present. The results could therefore suggest that growers may be aware of a PCN infestation and are choosing cultivars resistant to *G. rostochiensis* but they may have failed to have a species determination done and may be unaware that they have a population of *G. pallida*. It was also found that fewer cultivars resistant to G. *rostochiensis* were grown on sites free from PCN again indicating that when growers knew that they had no PCN problem, their choice of cultivar was based more on the most suitable cultivar for the market place.

The relationship between the species of PCN present and the population density for each site was investigated and it was found that sites that contained only *G. pallida* had

significantly higher population densities than sites that contained only *G. rostochiensis.* This would be expected since there were very cultivars grown with resistance to *G. pallida* (Table 2.11) and more cultivars grown with resistance to *G. rostochiensis.*

There was no significant relationship between the use of resistant cultivars and the mean population densities of the sites sampled. Although the sites that had cultivars grown with resistance to *G. pallida* had lower population densities, these were not statistically significant.

The standard cyst counts at Harper Adams revealed that 56% of the field samples tested contained PCN, but only 49% contained viable eggs. The second standard cyst count and bait plant tests at Rothamsted increased the detection of PCN by 8% to 64%. These results strongly suggest that many PCN infestations are cryptic due to inadequacies of conventional sampling and extraction methods, and detection can only be improved if testing beyond the routine counts is carried out. Turner (1993) found a positive linear relationship exits between the number of times a field was sampled and detection ($r = 0.992$) and recommended a single 360 g sample in fields up to 2 ha as the minimum practical and economic sampling intensity for PCN detection and certification purposes.

In the European Community, Council Directive 69/465//EEC on the control of PCN requires that seed potatoes for marketing are only produced on land that has been officially declared free from PCN infestation (Haydock & Evans, 1994). The approved methods of soil-sampling and nematode extraction for detection purposes are described in EPPO quarantine procedure No. 30 (Anon, 1991) where it is recommended that 100 cores, each of 4-5 ml, are taken from the top 5 cm of soil, distributed on a grid pattern throughout the

plot. This provides a total sample of approximately 500g which is processed completely in the laboratory (Anon, 1991). However, statutory soil sampling cannot guarantee complete freedom from PCN cysts (Haydock & Evans, 1994). The results are qualitative (i.e. present or absent) and population densities are of no importance for regulatory sampling (Haydock & Evans, 1994).

The EPPO recommendations (Anon, 1991) for soil sampling require a higher number of cores to be taken and a larger volume of soil to be processed than was done for this survey. They would therefore give a greater chance of detection of infestations especially at low population densities. Since more cores are taken, a new infestation which is in a very localised area in a field may well be detected by an EPPO sampling method but not by a less rigorous sampling method.

The samples collected for this survey were collected in a grid pattern and larger cores were taken so that more soil would be collected. The additional soil was not all extracted but was used in the bait tests and to provide a collection of PCN populations from a wide range of soil types in different geographical locations. The aims of this survey were to detect as many populations as possible, but also to provide sufficient cysts for species determination of the cysts present. The results from of the second extraction and bait plant test suggest that many infestations may be going unnoticed in commercial fields in their early stages. With the ever increasing problem of *G. pallida* with its slower decline rates, growers should sample more intensively to ensure that low levels of PCN are not allowed to increase to go undetected and to build up to damaging levels.

Another aim of this study was to compare IEF, ELISA and PCR-based techniques when used for determination of species of potato cyst nematodes in field samples, rather than PCN grown under controlled conditions with new cysts full of new eggs. This means that some of the cysts were of very poor quality and difficult to identity. All the methods were laborious, with no significant advantage of any one noted over any of the others. However, PCR and IEF results can be obtained in one day, whereas ELISA results are only obtained in two days. All of them appeared to distinguish the two species of PCN and all were also able to register population mixtures, but they frequently were not in full agreement. A greater number of positive results were obtained with PCR than with any other method, indicating the greater sensitivity of this method. All the 269 samples that were found to contain PCN were processed by PCR, while sufficient cysts were available in only 83 and 93 samples for processing by IEF and ELISA respectively. When the same samples were tested by all methods, some agreement was noted on the identification of samples of *G. rostochiensis*, but ELISA failed to agree with other methods on the identification of *G. pallida* and of mixed populations. This was due to the frequent uncertainty of cyst identification by ELISA (Appendix 6).

The disagreement between the results obtained in this study may be explained by differences in the principles of operation and the sensitivity of each of the methods. The sensitivity of PCR is much greater than isoelectric focusing, with the DNA from single eggs or juveniles sufficient for identification. Protein isoelectric focusing is very useful for PCN identification, but requires large numbers of viable eggs. Low viability cysts often contain insufficient protein for diagnosis (Fleming, Turner, Powers & Szalansky, 1998). The recent development of multiplex PCR tests may offer the way forward for nematode

identification. Multiplex PCR tests have also been used to identify other nematodes species: *Heterodera* (Fleming *et al.*, 1998) and *Meloidogyne* species (Zijlstra, 1997).

Both sets of PCR primers failed to yield amplification products from some field samples known to contain eggs. One of these sets (Mulholland *et al.*, 1996) also failed in previous work to detect PCN DNA from field samples known to contain live eggs (Evans *et al.*, 1998). Several factors may affect the amplification of DNA in PCR. These include the inhibition of DNA amplification at high concentrations of template DNA (Ibrahim, unpublished data), heterogeneity in the ITS sites (Bulman & Marshall, 1997), genetic differences within the species *G. pallida* (Fleming & Marks, 1983), genomic variation (Burrows *et al.*, 1996; Phillips, Blok, Ploeg & Harrower, 1998) or the presence of different pathotypes in the same sample (Kort *et al.*, 1977; Stone *et al.*, 1986). Even so, the potential sensitivity and specificity of the PCR and ELISA make them obvious choices for determination and quantification of PCN species in field populations. The promise of the ELISA-based system may be increased if better, more specific antibodies are found, perhaps by exploiting the phage display system. This would eliminate some of the uncertainties over cyst identification and thereby increase sensitivity. A phage display library targeted at plant parasitic nematodes would allow antibodies that recognise other common soil-dwelling nematodes to be found. For applications such as quarantine facilities, rapid tests would be of great value and are likely to be developed commercially in the future (Williamson, 1991).

3.0 Chapter 3.

Field Experiment One

The use of the soil fumigant 1,3-dichloropropene in combination with granular nematicides for the control of potato cyst nematodes

3.1 Introduction

As already discussed in Chapter 1, PCN cause substantial yield losses to potato crops each year. An integrated approach to PCN management using nematicides, rotations and resistant cultivars has been advocated for many years but the combinations of tactics that are advised must be soundly based. Granular nematicides are important in the control of PCN although they probably have been relied upon too heavily by growers (Evans & Haydock, 2000). They are more effective at reducing yield loss than controlling nematode population increase because PCN multiplication is density dependent and, at densities at which an economic yield return is obtained from nematicide use, the nematicides mainly kill nematodes that are surplus to the carrying capacity of the crop (Evans & Haydock, 2000). While the granular nematicides affect the hatched juveniles, fumigant nematicides such as 1,3-D kill PCN within the cysts. With very high PCN levels it is not unusual for growers to use a combination of 1,3-D followed by a granular nematicide. In the UK, the two most widely used granular nematicides are aldicarb (Temik) and oxamyl (Vydate) which are both oxime-carbamates and make up 49% and 44% of the market share respectively (Evans & Haydock, 2000). The remainder of the market is made up by two organophosphates: fosthiazate (Nemathorin) with 6% and ethoprophos (Mocap) with 1%. The costs of these chemicals are approximately £300-330 ha^{-1} and it is important that they are applied correctly (Evans & Haydock, 2000). Aldicarb, oxamyl and ethoprophos are approved for use as both a nematicide and an insecticide but fosthiazate is only approved for use as a nematicide (Whitehead, 1999). In 1998, approximately 1,250 ha were treated with fumigant nematicides at a cost of approximately £600 000 (Parker, 1999).

3.1.1 Aims

The aims of the experiment were to assess the effectiveness of use of 1,3-D in combination with three granular nematicides for the control of PCN and for an increase in yield. A field experiment was done using the nematicides aldicarb, oxamyl and fosthiazate.

The objectives to be determined were:

- whether 1,3-D in combination with a granular nematicide would control PCN multiplication more than either treatment used singly
- whether 1,3-D in combination with a granular nematicide would increase yields more than either treatment used singly
- whether 1,3-D in combination with a granular nematicide would reduce plant root invasion more than either treatment used singly

3.2 Materials and methods

3.2.1 Experimental design

The experiment had a two by five factorial design. There were two levels for autumn fumigation, either treated or untreated, and these were combined with five spring treatments. The factorial design enabled comparisons to be made between the granular nematicides singly and in combination with 1,3-D, and between autumn and spring fumigation with 1,3-D. The ten treatments are shown in Table 3.1.

Table 3.1. *List of treatments for field experiment one*

Treatment	Autumn 1997	Spring 1998
1	untreated	untreated
2	untreated	aldicarb (3.36 kg a.i. ha^{-1})
3	untreated	oxamyl (5.5 kg a.i. ha^{-1})
4	untreated	fosthiazate (3.0 kg a.i. ha^{-1})
5	untreated	spring 1,3-D (211.5 l a.i. ha^{-1})
6	1,3-D (211.5 l a.i. ha^{-1})	untreated
7	1,3-D (211.5 l a.i. ha^{-1})	aldicarb (3.36 kg a.i. ha^{-1})
8	1,3-D (211.5 l a.i. ha^{-1})	oxamyl (5.5 kg a.i. ha^{-1})
9	1,3-D (211.5 l a.i. ha^{-1})	fosthiazate (3.0 kg a.i. ha^{-1})
10	1,3-D (211.5 l a.i. ha^{-1})	spring 1,3-D (211.5 l a.i. ha^{-1})

The plot layout is shown in Fig. 3.1. The plot number is indicated on the top row of each square. The plots were three beds wide (5.5 m) and nine metres long. Since one bed is made up from two rows of potatoes, each plot was six rows wide. The middle two rows in each plot were used as the harvest rows. The ten treatments were replicated in each of the five blocks. The blocks were arranged along the length of the experiment. Blocks 1, 3 and 5 are shaded in grey and blocks 2 and 4 are unshaded.

Blocks

1	2	3	4	5
10 1,3-D + oxamyl	**20** 1,3-D + aldicarb	**30** untreated + untreated	**40** 1,3-D + 1,3-D	**50** untreated + aldicarb
9 untreated + oxamyl	**19** untreated + 1,3-D	**29** untreated + fosthiazate	**39** 1,3-D + untreated	**49** untreated + untreated
8 untreated + 1,3-D	**18** untreated + oxamyl	**28** 1,3-D + untreated	**38** 1,3-D + fosthiazate	**48** untreated + 1,3-D
7 untreated + fosthiazate	**17** 1,3-D + fosthiazate	**27** 1,3-D + 1,3-D	**37** untreated + aldicarb	**47** untreated + fosthiazate
6 untreated + aldicarb	**16** untreated + fosthiazate	**26** untreated + oxamyl	**36** 1,3-D + oxamyl	**46** 1,3-D + aldicarb
5 1,3-D + untreated	**15** 1,3-D + 1,3-D	**25** 1,3-D + fosthiazate	**35** untreated + oxamyl	**45** 1,3-D + oxamyl
4 1,3-D + aldicarb	**14** untreated + aldicarb	**24** 1,3-D + aldicarb	**34** untreated + untreated	**44** 1,3-D + 1,3-D
3 untreated + untreated	**13** 1,3-D + oxamyl	**23** untreated + 1,3-D	**33** 1,3-D + aldicarb	**43** 1,3-D + untreated
2 1,3-D + fosthiazate	**12** 1,3-D + untreated	**22** untreated + aldicarb	**32** untreated + 1,3-D	**42** 1,3-D + fosthiazate
1 1,3-D + 1,3-D	**11** untreated + untreated	**21** 1,3-D + oxamyl	**31** untreated + fosthiazate	**41** untreated + oxamyl

9 m

(3 beds, 5.5 m)

Fig. 3.1. Plot layout for Field Experiment One: The use of 1,3-D in combination with three granular nematicides for the control of potato cyst nematodes

3.2.2 Selection of experimental site

A site for the experiment was chosen in Common Field, which is part of Harper Adams University College farm. The topsoil is a stoneless or slightly stony loamy sand and the subsoil is a permeable stoneless or very slightly stony reddish loamy sand with accumulations of iron and inorganic material over sandstone at about 50 cm deep (Beard, 1988). The site had ADAS nutrient indices (Anon, 1994) of P = 4, K = 3, Mg = 2, and a pH of 7.4.

This site for the experiment was selected in early 1997. The site was already infested with PCN but a higher initial population density was required for the experiment since a fumigant and a granular nematicide were going to be used in combination in some of the treatments. It was therefore decided to grow a crop of susceptible Désirée potatoes in 1997 to increase the population densities.

After the Désirée crop was grown, population densities were estimated (3.2.4) for nine plots selected from across the site to check whether the site was suitably infested for the experiment. The results (Fig. 3.2) show that all of the plots tested were infested with PCN. The Pi's ranged from 34 to 127 eggs g^{-1} soil and the mean population density was 66 eggs g^{-1} soil. The site was therefore deemed suitable.

A check of PCN species identity by IEF (Fleming & Marks, 1983) showed that both species of PCN were present but that the most abundant was *G. rostochiensis*.

Blocks

1	2	3	4	5
10	**20**	**30**	**40**	**50**
9 35 eggs g^{-1} soil	**19**	**29** 64 eggs g^{-1} soil	**39**	**49** 34 eggs g^{-1} soil
8	**18**	**28**	**38**	**48**
7	**17**	**27**	**37**	**47**
6	**16**	**26**	**36**	**46**
5 73 eggs g^{-1} soil	**15**	**25**	**35**	**45** 89 eggs g^{-1} soil
4	**14**	**24** 41 eggs g^{-1} soil	**34**	**44**
3	**13**	**23**	**33**	**43**
2	**12**	**22** 49 eggs g^{-1} soil	**32**	**42** 127 eggs g^{-1} soil
1 34 eggs g^{-1} soil	**11**	**21**	**31**	**41**

Fig. 3.2. Preliminary initial population densities (eggs g^{-1} soil) of plots tested

3.2.3 Sampling soil for PCN

The plots were marked out for the experiment on 24 October 1997. The application of 1,3-D required that the ground be flat. The initial PCN population densities (Pi) for each plot were estimated from samples taken on the 27/28 October 1997 using a "cheese-corer" style auger with a half-cylindrical blade. Sixty cores (1.5 cm × 10 cm) were taken from the soil that would form the middle two rows (i.e. the harvest rows) in each plot and bulked to form one soil sample. The final population densities (Pf) were estimated from samples

taken on the 11 September 1998 by bulking 60 cores from the soil that had formed the middle two rows in each plot. The soil had been mixed at harvesting using a tractor mounted, single row potato spinner (3.2.16).

3.2.4 Cyst detection and estimation of cyst contents

The soil samples were collected in linen bags and were air-dried at 25°C for at least 5 days. The samples were sieved through a 4 mm aperture sieve, thoroughly mixed and a 200 g sub-sample taken from each for cyst extraction using a Fenwick can (Fenwick, 1940). The numbers of PCN cysts present in each extract were counted under a stereomicroscope and 50 cysts were removed for an egg count. The cysts were soaked in water overnight before crushing to release the eggs. The resulting suspension was made up to 50 ml and the number of eggs present in a 1 ml aliquot counted. From the counts made, a population density in eggs g^{-1} of dried field soil was calculated for each sample (Southey, 1970).

3.2.5 Species identification

The species of PCN present were determined by iso-electric focusing (IEF), using 50 cyst samples in the procedure of Fleming & Marks (1983). The cysts were taken from a random selection of plots across the experimental site.

3.2.6 Application of fumigant

The previous crop on the experimental site was Désirée, which was harvested on 22 October 1997. The site was power-harrowed and deep cultivated with a shakaerator (Commando Series 2 shakaerator; McConnel, Ludlow, England) to loosen the soil on 23 October 1997.

There was both an autumn and a spring application of 1,3-D. For the autumn treatment, plots were treated on 29 October 1997 at a depth of 15 cm and at a soil temperature of 7.5°C. (The soil temperature should be a minimum of 5°C for the liquid to form a gas once it is injected into the soil). The injection of 1,3-D (as Telone II: 94% a.i. w/w; Dow Agrosciences) at 211.5 litres a.i. ha^{-1} was done using a Rumpstadt Combiject (Rumpstadt, Haringvliet, The Netherlands) which also sealed the surface using a powered-roller. The width treated by the machine was 3 m, so two passes were made to treat each plot. Since the plots were 5.5 m wide, the area treated was 0.5 m wider than the plots. However, since each plot was beside a tramline, the excess area treated was able to be part of a tramline and therefore did not affect adjacent plots.

After treatment with 1,3-D, the site was left undisturbed over winter. The site was then ploughed to 25 cm depth and shakaerated on 27 February 1998 in preparation for the spring application of 1,3-D. The spring application of 1,3-D was made on the 16 March 1998 at 15 cm deep and at a soil temperature of 6.5°C. After application, the site was left undisturbed until the 6 April, when it was chisel-ploughed to release any gas remaining in the soil.

3.2.7 The effect of 1,3-D on growth and germination of weed seeds

The plots used for assessments and the soil used in germination experiments were made on the twenty plots that made up the following four treatments: 1 (untreated), 5 (spring application of 1,3-D), 6 (autumn application of 1,3-D) and 10 (autumn and spring application of 1,3-D). Assessments were made over a period of several months and some were made before the spring 1,3-D had been applied. This effectively meant that there were only two treatments: 1 and 5 were untreated and 6 and 10 were treated with 1,3-D,

and this is how the results were analysed and presented. After the spring application of 1,3-D, the four treatments were analysed and presented separately.

3.2.7.1 Germination of weed seeds in soil after application of 1,3-D

During the application of 1,3-D, the ground was rolled to seal the surface and this resulted in a smooth flat surface which was left undisturbed over winter. The numbers of weeds that had germinated and were growing were counted on the 11 December 1997 (43 days after autumn fumigation). This was done using a square quadrat of side 0.25 m and sixteen assessments were made on each plot.

3.2.7.2 Percentage of ground covered by weeds

The percentage of ground covered by weeds was assessed on 18 February 1998 (112 days after autumn fumigation). A square quadrat of side 1 m was used and eight assessments were made on each plot.

3.2.7.3 Weed seed germination in soil treated with 1,3-D

The effect of 1,3-D on the weed seed bank was determined by assessing the germination of seeds from both treated and untreated soil. Counts were taken of the numbers of seeds that germinated under glasshouse conditions using a protocol similar to that of Roberts & Neilson (1981). Soil samples consisting of 12 cores (20 cm × 3.5 cm) were taken from each plot to give approximately 2.5 kg of soil. The soil was thoroughly mixed and sieved and 2 kg was weighed into trays 30 cm long by 15 cm wide. The depth of soil in each tray was approximately 5 cm. The soil was mixed periodically to bring more seeds nearer to the surface and increase their likelihood of germination.

Two sets of soil samples were collected. The first set of soil samples was collected on the 25 February 1998 (119 days after autumn fumigation) and the second set was collected on the 21 April 1998 (174 days after autumn fumigation; 36 days after spring fumigation).

The numbers of germinating weeds were counted and removed as they appeared. This was done for the first set of samples on 24 March; 4, 14, 24 April; 12, 25 May; 25, 31 July; 8 October and 2 November. This was done for the second set of samples on 27 May; 11, 25, 31 July; 8 October and 2 November.

3.2.8 Application of granular nematicides

The experimental site was ridged up to form the beds, which were then bed-tilled and stone-separated on 10 May. The granular nematicides were applied to the beds using a land-wheel-metered granule distributor on the day of planting. The nematicides used were: aldicarb (as Temik 10G; 10% a.i. w/w; Rhone Poulenc) applied as granules at 3.36 kg a.i. ha^{-1}; fosthiazate (as Nemathorin; 10% a.i. w/w; Zeneca) applied as granules at 3.0 kg a.i. ha^{-1} and oxamyl (as Vydate 10G; 10% a.i. w/w; DuPont) applied as granules at 5.5 kg a.i. ha^{-1}. The nematicides were incorporated to a depth of 15cm using a tractor mounted rotavator.

3.2.9 Crop management

Before planting and after soil analysis, fertiliser was applied as required and the crop was managed according to standard agrochemical practices for the control of weeds and diseases (Appendix 8).

3.2.10 Cultivar planted

Estima was chosen as it is a widely grown commercial cultivar with no resistance to either species. This enabled differences in PCN multiplication (Pf/Pi ratios) between treatments to be measured. Estima (Super Elite 2, size grade: 50-55 mm) was planted at 20-25 cm depth and 28 cm spacing on 11 May using a tractor mounted potato planter.

3.2.11 Number of weeds growing on potato ridges

A second assessment on the numbers of weeds that had germinated and were growing was made on the 27 May 1988 (210 days after autumn fumigation and 62 days after spring fumigation). The assessment was made 16 days after planting on the top of the potato ridges. At this time, the potato plants had not yet fully emerged and were not having any competitive effect on the weed population. A square quadrat of side 0.25 m was again used and eight assessments were made on each plot.

3.2.12 Plant emergence

The numbers of plants emerged from the middle two rows in each plot were counted at 23, 29, 35 and 47 days after planting. The plant was defined as having emerged when any part of the shoot was visible when viewed vertically from above the planting station (Bastiman, Bevis & Wellings, 1985).

3.2.13 Percentage ground cover

The percentage ground cover was measured at 29, 35 and 50 days after planting using a grid 90 cm wide by 60 cm long. The grid was divided into 100 squares, each 9 cm by 6 cm. The grid was placed over a row of plants from the middle bed in the plot and the number of squares in which more than 50% of the ground was covered by green leaves were counted

(Burstall & Harris, 1983). Two readings were taken for each of the 2 rows in the middle bed. The first reading was taken approximately 3 metres into the plot and the subsequent reading was taken 2 metres from the first.

3.2.14 Root invasions

Two plants were taken from each plot at 44 days after planting to measure root invasion. One plant was taken from each of the inner rows of the two non-harvest beds to avoid disrupting the two rows which formed the middle bed, in which yield would be measured. Each plant was systematically selected by choosing the 12^{th} plant from the end of the plot. The roots were chopped into sections of 2 cm or less, mixed and a 2 g sub-sample taken for each plot. The sample was placed in formal acetic alcohol (FAA) to preserve the roots until root invasion was assessed (Hooper, 1986). The 2 g sub-sample was stained in acid fuchsin (Bridge, Page & Jordan, 1982) and the numbers of developing juveniles were counted.

3.2.15 Incidence of *R. solani*

The plants assessed for *R. solani* were taken from the 10 plots that made up treatments 1 and 6. Five plants were collected from each plot for assessment at 50 days after planting. The plants were randomly selected from the two outer rows of the non-harvest beds and an assessment was made using a key described by Simons & Gilligan (1997). Each plant was examined to determine the number of stems, incidence of stem canker (proportion of infected stems per plant) and severity of stem canker on each stem. Stems were assigned a severity score of 1-4 which were:

1. no stem canker
2. up to one-third of the stem length affected by the lesion
3. one- to two-thirds of the length affected

4. more than two-thirds of the length affected

A weighted estimate of disease severity was computed for each plant as $(3X_i\ W_i)\ /(33X_i)$ in which X_i is the number of stems in each of the four categories and W_i takes the value 0,1,2,3 for $i = 1, 2, 3, 4$ (Simons & Gilligan, 1997).

3.2.16 Harvesting and grading

The experiment was desiccated at 91 days after planting using glufosinate-ammonium (as Challenge: 150 g l^{-1}, AgrEvo) and harvested at 121 days after planting. The ends of the plot were harvested by hand using a fork and a 5 m length was harvested from the two middle rows in each plot using a tractor mounted, single row potato spinner. The mechanically harvested plots were then hand forked to ensure that all tubers had been removed.

Tubers were mechanically graded into four size fractions: less than 45 mm, 45-65 mm, 65-85 mm and greater than 85 mm. The number of tubers was counted and weighed for each grade.

3.2.17 Statistical analysis, data handling and presentation of results

The data from the experiment were analysed using Genstat™ 5, Release 4.1, (Lawes Agricultural Trust, IACR-Rothamsted, UK). All data were checked to establish whether they had normal distributions. A general analysis of variance was done where possible on untransformed data but the data were transformed when necessary. An analysis of variance with a covariate was also used when appropriate. When there was no significant interaction between fumigation and granular nematicide treatment, significant results are shown in the text and a complete list of results are in Appendix 11.

3.3 Results

3.3.1 Assessment of weed numbers

3.3.1.1 Germination of weed seeds in soil after application of 1,3-D

The results for the number of weeds on 11 December 1997 (43 days after autumn fumigation) in plots treated and untreated with 1,3-D are in Table 3.2. The number of weeds germinating in untreated plots is nearly six-fold greater than in treated plots, a significant difference ($P < 0.001$). This is clearly seen in Plates 3.1 and 3.2.

Table 3.2. *The number of weeds (weeds/m^2) in plots treated and untreated with 1,3-D on 11 December 1997 (43 days after autumn fumigation)*

Treatment	-1,3-D	+1,3-D
Number of weeds /m^2	6353	1099

SED	Significance (P =)	df	CV %
450.0	<0.001	14	27.0

Key finding

- fumigation with 1,3-D significantly reduced the number of weeds germinating in treated plots by nearly six-fold

Plate 3.1. View of an untreated plot showing many weed seeds germinating

Plate 3.2. View of a plot treated with 1,3-D showing few weed seeds germinating

3.3.1.2 Percentage of ground covered by weeds

The percentages of ground covered by weeds on 18 February 1998 (112 days after autumn fumigation) in plots treated and untreated with 1,3-D are in Table 3.3. The ground covered by weeds in treated plots was significantly lower ($P < 0.001$) than in untreated plots. This is clearly seen in Plate 3.3.

Table 3.3. *The percentage of ground covered by weeds (after angular transformation) on 18 February 1998 (112 days after autumn fumigation) in plots treated and untreated with 1,3-D (untransformed data in parentheses)*

Treatment	-1,3-D	+1,3-D
Ang (% ground cover)	40.3 (41.8)	17.0 (8.6)

SED	Significance (P =)	df	CV %
1.008	<0.001	14	7.9

Key finding

- fumigation with 1,3-D significantly reduced the percentage of ground covered by weeds

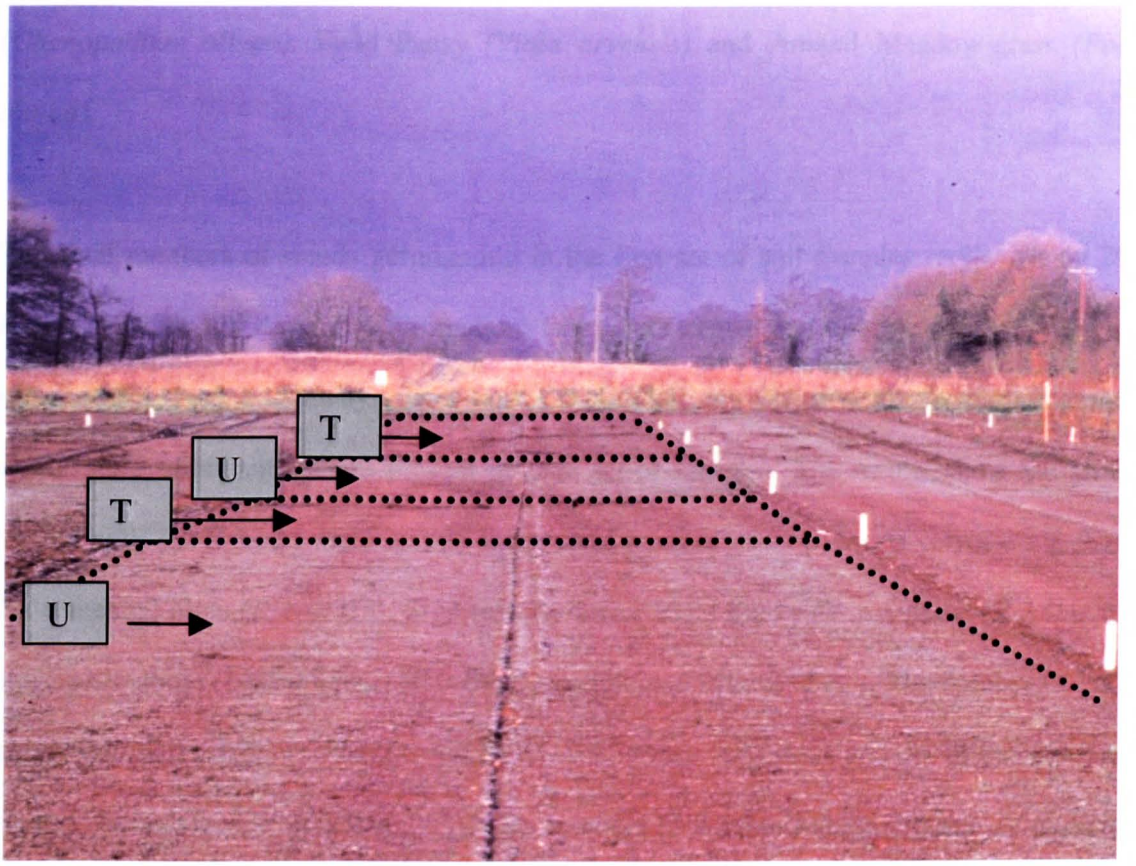

Plate 3.3. Weed growth in treated (T) and untreated (U) plots. The dotted lines indicate plot boundaries. The untreated plots have a high ground covering of weeds while the plots treated with 1,3-D have a lower ground covering of weeds

3.3.1.3 Weed seed germination in soil treated with 1,3-D

The species and total numbers of weed seeds germinating under glasshouse conditions in soil from plots treated and untreated with 1,3-D are shown in Table 3.4. Plate 3.4 shows the trays containing weeds that had germinated. In total, 23 species of broad-leaved weeds and grasses germinated in the soil collected. The most common were Fat-hen *(Chenopodium album),* Field Pansy *(Viola arvensis)* and Annual Meadow-grass *(Poa annua).*

The total numbers of weeds germinating in the first set of soil samples (collected on 25 February 1998, 119 days after fumigation) from plots treated and untreated with 1,3-D are in Table 3.5. Statistical analysis was done on the total number of weeds germinating and on the most abundant species present in each set of samples. The results show the plots treated with 1,3-D had a significantly lower total number of weed seeds germinating than the untreated plots ($P = 0.018$). Significantly fewer Field Pansy *(V. arvensis)* ($P = 0.006$) and Small Nettle *(Urtica urens)* ($P = 0.007$) germinated in treated soil. The number of Fat-hen *(C. album)* seedlings was less in treated than in untreated plots and the difference was close to being significant ($P = 0.063$). The numbers of Annual Meadow-grass *(P. annua),* Black-bindweed *(Fallopia convolvulus)* and Chickweed *(Stellaria media)* were also decreased but the differences were not significant.

The numbers of weeds germinating in the second set of samples collected on 21 April 1988 (174 days after autumn fumigation and 36 days after spring fumigation) from soil treated and untreated with 1,3-D are in Table 3.6. The greatest numbers of weeds were found in the untreated plots and the fewest in the plots that had been treated with both an autumn and a spring application of 1,3-D, with numbers in plots that had either an autumn or a

spring application intermediate. Although the results show a clear trend for a reduction in weed numbers after treatment by 1,3-D, the differences were not significant ($P = 0.099$). However, the number of Field Pansy *(V. arvensis)* was significantly reduced ($P = 0.010$). The numbers of Fat-hen *(C. album)* and Annual Meadow-grass *(P. annua)* were also decreased but not significantly.

Key findings

- significantly fewer weeds germinated in soil that had been fumigated with 1,3-D
- significantly fewer numbers of Field Pansy *(V. arvensis)* and Small Nettle *(U. urens)* germinated in treated soil

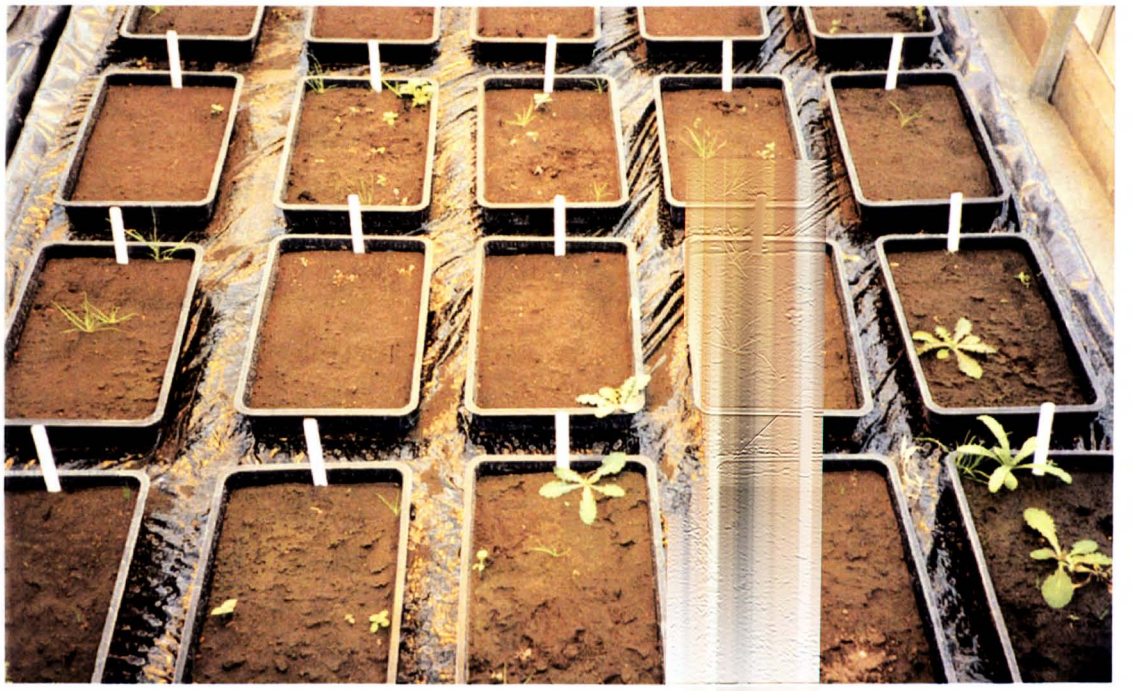

Plate 3.4. View of weeds germinating in soil under glasshouse conditions collected from plots treated and untreated with 1,3-D

Table 3.4. *The total number and species of weed seeds germinating from all plots treated and untreated with 1,3-D*

Weed species	No. of weeds in first set of samples	No. of weeds in second set of samples
Broad-leaved weeds		
Annual Sowthistle *(Sonchus* spp.)	7	2
Black Nightshade *(Solanum nigrum)*	12	0
Black-bindweed *(Fallopia convolvulus)*	30	7
Bugloss *(Anchusa arvensis)*	3	0
Chickweed *(Stellaria media)*	50	11
Common Field-speedwell *(Veronica persica)*	1	0
Corn Mint *(Mentha arvensis)*	3	0
Fat-hen *(Chenopodium album)*	450	277
Field Pansy *(Viola arvensis)*	370	46
Fumitory *(Fumaria officinalis)*	0	6
Groundsel *(Senecio vulgaris)*	10	56
Ivy-leaved Speedwell *(Veronica raphanistrum)*	1	0
Knotgrass *(Polygonum aviculare)*	7	1
Orache *(Atriplex patula)*	4	0
Perennial Sowthistle *(Sonchus arvensis)*	1	9
Rayless Mayweed *(Chamomilla suaveolens)*	0	1
Redshank *(Polygonum persicaria)*	33	14
Runch *(Raphanus raphanistrum)*	1	1
Scarlet Pimpernel *(Anagallis arvensis)*	7	1
Shepherd's-purse *(Capsella bursa-pastoris)*	8	0
Small Nettle *(Urtica urens)*	64	22
Grass species		
Annual Meadow-grass *(Poa annua)*	95	36
Rough Meadow-grass *(Poa trivialis)*	1	0
Total	1158	490

Table 3.5. *The number of weeds (number of weeds/ 2 kg sample) germinating in soil collected on 25 February 1998 (119 days after fumigation) from plots treated and untreated with 1,3-D*

Treatment	-1,3-D	+1,3-D
Total number of weeds	85.3	49.2
Field Pansy *(V. arvensis)*	24.8	12.2
Small Nettle *(U. urens)*[a]	1.52 (4.8)	0.71 (1.6)
Fat-hen *(C. album)*[a]	2.66 (26.9)	2.00 (18.1)
Annual Meadow-grass *(P. annua)*[a]	1.58 (5.2)	1.17 (4.3)
Black-bindweed *(F. convolvulus)*[a]	0.84 (1.7)	0.70 (1.3)
Chickweed *(S. media)*[b]	2.9	2.1

	SED	Significance (P =)	df	CV %
Total number of weeds	13.43	0.018	14	44.6
Field Pansy *(V. arvensis)*	3.91	0.006	14	47.2
Small Nettle *(U. urens)*	0.255	0.007	14	51.0
Fat-hen *(C. album)*	0.323	NS	14	31.0
Annual Meadow-grass *(P. annua)*	0.404	NS	14	65.6
Black-bindweed *(F. convolvulus)*	0.272	NS	14	79.3

[a] values were transformed by natural logarithm, untransformed data in parentheses
[b] data was skewed and no suitable transformation could be found, therefore no statistical analysis was done

Table 3.6. *The natural logarithm of the number of weeds (number of weeds/ 2 kg sample) germinating in soil collected on 21 April 1988 (174 days after autumn fumigation; 36 days after spring fumigation) treated and untreated with 1,3-D (untransformed data in parentheses)*

Treatment	untreated	spring 1,3-D	autumn 1,3-D	autumn + spring 1,3-D
Log_e (total no. of weeds)	3.50 (36)	2.65 (22)	2.97 (27)	2.42 (19)
Field Pansy *(V. arvensis)*	1.74 (5.4)	0.74 (1.6)	0.72 (1.2)	0.55 (1.0)
Fat-hen *(C. album)*	2.22 (14.2)	1.71 (8.4)	2.23 (19.2)	1.87 (13.6)
Annual Meadow-grass *(P. annua)*	1.11 (2.20)	1.13 (3.00)	0.50 (1.00)	0.64 (1.00)
Groundsel *(S. vulgaris)*[a]	(6.0)	(2.0)	(2.0)	(1.2)

	SED	Significance ($P =$)	df	CV %
Log_e (total no. of weeds)	0.406	NS	12	22.2
Field Pansy *(V. arvensis)*	0.312	0.010	12	52.6
Fat-hen *(C. album)*	0.381	NS	12	30.0
Annual Meadow-grass *(P. annua)*	0.405	NS	12	75.8

[a] data was skewed and no suitable transformation could be found, therefore no statistical analysis was done

3.3.1.4 Number of weeds growing on potato ridges

The number of weeds growing on 27 May 1988 (210 days after autumn fumigation; 62 days after spring fumigation) on potato ridges in plots treated and untreated with 1,3-D is shown in Table 3.7. The results show that the greatest numbers of weeds were found in the untreated plots. There were clearly fewer weeds in plots that had an application of 1,3-D, although the differences were not significant. The plots that had both a spring and an autumn application of 1,3-D contained the smallest numbers of weeds.

Table 3.7. *The natural logarithm of the number of weeds on 27 May 1988 (210 days after autumn fumigation; 62 days after spring fumigation) on potato ridges in plots treated and untreated with 1,3-D (untransformed data in parentheses)*

Treatment	untreated	spring 1,3-D	autumn 1,3-D	autumn + spring 1,3-D
Log_e	4.40	4.02	3.90	3.69
(no. of weeds /m^2)	(112)	(63)	(69)	(50)

SED	Significance (P =)	df	CV %
0.497	0.569	14	17.6

Key findings

- both autumn and spring fumigation with 1,3-D reduced the number of weeds germinating on potato ridges although the results were not statistically significant

3.3.2 Plant emergence

Plant emergence was measured at 23, 29, 35 and 47 days after planting and the results are shown in Table 3.8. An analysis of variance for repeated measurements was done to assess the effect of treatments over time. The time analysis shows that there was a significant effect of 1,3-D on plant emergence over time ($P < 0.001$). 1,3-D significantly increased the emergence at 23, 29 and 35 days after planting but by 47 days after planting, the numbers of plants emerged were not affected by treatment with autumn 1,3-D.

The time analysis shows that there was a significant effect of spring treatment over time ($P < 0.01$). At 23 days after planting, plant emergence was significantly increased by the application of aldicarb and oxamyl ($P = 0.022$) but not by fosthiazate or the spring application of 1,3-D. There was no interaction between the autumn and spring treatments. At 29 days after planting, all three granular nematicides had a significant effect ($P = 0.048$) on plant emergence but at 35 and 47 days after planting the effect of the three granular nematicides was not seen. The time analysis also shows that there was no significant interaction between autumn and spring treatments over time.

Key findings

- plant emergence was significantly advanced by the autumn application of 1,3-D and by all of the granular nematicides
- the final emergence of plants was not significantly different between treatments

Table 3.8. *The effects of fumigation and granular nematicide treatment on the percentage of potato plants emerged at 23, 29, 35 and 47 days after planting (DAP)*

	DAP			
	23	29	35	47
Spring means[a]				
untreated	19.4	39.3	58.4	76.7
aldicarb	31.1	54.1	62.4	75.1
oxamyl	33.8	50.4	60.5	72.3
fosthiazate	28.0	52.4	62.1	73.7
spring 1,3-D	23.5	47.6	62.5	74.8
1,3-D means[b]				
-D	20.6	41.2	55.5	73.9
+D	33.7	56.3	66.8	75.2

	SED	Significance (P =)	df	CV %
23 DAP				
+/-D	6.42	NS	36	
spring means[a]	4.54	0.022	36	
1,3-D means[b]	2.87	<0.001	36	37.4
29 DAP				
+/-D	7.12	NS	36	
spring means	5.04	0.048	36	
1,3-D means	3.19	<0.001	36	23.1
35 DAP				
+/-D	6.24	NS	36	
spring means[a]	4.41	NS	36	
1,3-D means[b]	2.79	<0.001	36	16.1
47 DAP				
+/-D	4.52	NS	36	
spring means	3.19	NS	36	
1,3-D means	2.02	NS	36	9.6
Time analysis				
Time*1,3-D means	2.729	<0.001	80	
Time*spring means	4.315	<0.01	80	
Time*1,3-D means *spring means	6.102	NS	80	12.6

[a]means for treatments receiving spring or autumn treatment with 1,3-D

[b]means of all spring treatments that had the same autumn treatment

3.3.3 Percentage ground cover

Percentage ground cover was measured at 29, 35 and 50 days after planting (Table 3.9). The individual analysis of variance for each assessment date shows that the autumn application of 1,3-D significantly increased the ground cover at 29, 35 and 50 days after planting. An analysis of variance for repeated measurements was done to assess the effect of treatment over time. The time analysis shows that there was a significant effect of 1,3-D of ground cover over time ($P < 0.001$). It was found that 1,3-D significantly increased the percentage ground cover only at 35 and 50 days after planting indicating that the effect of 1,3-D in increasing ground cover increased over time.

The time analysis shows that there was no significant effect of spring treatment over time. The individual analyses shows that only oxamyl significantly increased ground cover at 29 days after planting ($P = 0.028$) and at 35 days after planting ($P = 0.043$). At 50 days after planting there was no significant effect from any of the spring treatments. The time analysis also shows that there was no interaction between the autumn and spring treatments over time. The differences in percentage ground cover between treatments are shown in Plate 3.5. In addition the mean plant emergence was found to be significantly correlated to the mean ground cover ($P < 0.001$) with a r^2 value of 0.533.

Key findings

- at 29 days after planting, the percentage ground cover was significantly increased by both the autumn application of 1,3-D and oxamyl
- by 50 days after planting, only the autumn application of 1,3-D significantly increased the percentage ground cover

Plate 3.5. Percentage ground cover in Field Experiment One. The plot in the foreground was treated only with oxamyl while the plot behind was treated with both a spring and an autumn application of 1,3-D

Table 3.9. *The effects of fumigation and granular nematicide treatment on the percentage ground cover of potato plants at 29, 35 and 50 days after planting (DAP)*

	DAP		
	29	35	50
Spring means[a]			
untreated	1.5	8.1	50.4
aldicarb	2.4	11.0	58.5
oxamyl	3.5	13.3	60.0
fosthiazate	2.5	10.0	56.5
spring 1,3-D	2.5	9.6	55.9
1,3-D means[b]			
-D	1.4	7.0	45.8
+D	3.5	13.8	66.8

	SED	Significance (P =)	df	CV %
29 DAP				
+/-D	0.81	NS	36	
spring means[a]	0.57	0.028	36	
1,3-D means[b]	0.36	<0.001	36	51.5
35 DAP				
+/-D	2.33	NS	36	
spring means	1.64	0.043	36	
1,3-D means	1.04	<0.001	36	35.3
50 DAP				
+/-D	7.04	NS	36	
spring means	4.98	NS	36	
1,3-D means	3.15	<0.001	36	19.8
Time analysis				
Time*spring means	3.107	NS	105	
Time*1,3-D means	1.965	<0.001	105	
Time*1,3-D means *spring means	4.394	NS	105	27.2

[a b]see Table 3.8

3.3.4 Root invasion

The results for the root invasion assessments at 44 days after planting are in Table 3.10. The values were found to be skewed and were therefore transformed by taking natural logarithms. Root invasion was significantly decreased by the autumn application of 1,3-D ($P < 0.001$) and by all three granular nematicides ($P < 0.001$) but not by the spring application of 1,3-D. Overall, aldicarb and oxamyl resulted in significantly less root invasion than fosthiazate.

Table 3.10. *The effects of fumigation and granular nematicide treatment on Log_e root invasion (no. of juveniles g^{-1} root) of potato plants at 44 days after planting (DAP) (untransformed data in parenthesis)*

Treatment	-D	+D	spring means[a]
untreated	7.54 (2150)	6.13 (760)	6.84 (1455)
aldicarb	5.62 (360)	4.55 (110)	5.08 (235)
oxamyl	5.48 (280)	4.41 (110)	4.95 (195)
fosthiazate	6.74 (1240)	4.81 (220)	5.78 (730)
spring 1,3-D	7.38 (1830)	6.20 (640)	6.79 (1235)
1,3-D means[b]	6.55 (1172)	5.22 (368)	

	SED	Significance (P =)	df	CV %
root invasion				
+/-D	0.481	NS	36	
spring means[a]	0.340	<0.001	36	
1,3-D means[b]	0.215	<0.001	36	12.9

[a b]see Table 3.8

Key finding

- root invasion was significantly decreased by the autumn application of 1,3-D and by all three granular nematicides

3.3.5 Incidence of *R. solani*

The results for the assessments of *R. solani* are shown in Table 3.11. Although there was no significant difference in levels of *R. solani* between the two treatments, the plots that were treated with 1,3-D had a lower incidence of disease.

Table 3.11. *The effects of soil fumigation with 1,3-D on the incidence of* Rhizoctonia solani *(per plant) on the potato cultivar Estima at 50 days after planting*

Treatment	-1,3-D	+1,3-D
incidence	0.409	0.325

SED	Significance (P =)	df	CV %
0.0425	0.121	1	18.3

3.3.6 Tuber yield

The results for effects of fumigation and granular nematicide treatment on yield (t ha^{-1}) of individual grades of potato tubers at harvest (121 days after planting) are in Table 3.12. The weight of tubers less than 45 mm was significantly decreased (P = 0.031) by aldicarb

and oxamyl only. The weight of tubers 45-65 mm was significantly increased ($P < 0.001$) by the autumn application of 1,3-D. The weight of tubers 65-85 mm was significantly increased by the autumn application of 1,3-D ($P < 0.001$) and by all three individual granular nematicides ($P < 0.001$), but not by the spring application of 1,3-D.

The effects of fumigation and granular nematicide treatment on ware and total yields of potato tubers (t ha^{-1}) and the percentage of tubers which were of ware grade are shown in Tables 3.12 and 3.13. The ware yield was significantly increased by the autumn application of 1,3-D ($P < 0.001$) and by the all the spring treatments ($P < 0.001$). Oxamyl produced significantly higher yields than fosthiazate or the spring application of 1,3-D. Total yield was also significantly increased by the autumn application of 1,3-D ($P < 0.001$) and by all the spring treatments ($P < 0.002$). The yield from oxamyl treatment was significantly higher than that from fosthiazate or the spring application of 1,3-D.

The percentage of tubers that were of ware grade was significantly increased by the autumn application of 1,3-D ($P < 0.001$) and by the all the spring treatments ($P < 0.001$) with yields from oxamyl plots significantly higher than those from the spring application of 1,3-D (Table 3.13). There was a significant interaction ($P = 0.048$) between the autumn and spring treatments. The autumn application of 1,3-D significantly increased the percentage ware yield when plots were untreated in spring or received the spring application of 1,3-D, but not with any of the granular nematicides. The mean tuber weight (Table 3.13) was significantly increased by the autumn application of 1,3-D ($P < 0.001$) and by all of the granular nematicides ($P < 0.001$) but not by the spring application of 1,3-D.

Table 3.12. *The effects of fumigation and granular nematicide treatment on yield (t ha^{-1}) of individual grades of potato tubers and of ware and total yields at harvest at 121 days after planting*

	< 45 mm	45-65 mm	65-85 mm	ware	total
spring means[a]					
untreated	5.8	23.0	6.2	29.1	34.9
aldicarb	4.7	25.7	11.9	37.7	42.5
oxamyl	4.5	26.1	14.6	41.3	45.7
fosthiazate	4.9	25.6	9.6	35.2	40.1
spring 1,3-D	5.4	25.4	9.1	34.6	40.1
1,3-D means[b]					
-D	5.2	22.3	6.7	29.0	34.3
+D	4.9	28.1	13.9	42.2	47.1

	SED	Significance (P =)	df	CV %
< 45 mm				
+/-D	0.64	NS	36	
spring means[a]	0.45	0.031	36	
1,3-D means[b]	0.29	NS	36	19.9
45-65 mm				
+/-D	2.37	NS	36	
spring means	1.67	NS	36	
1,3-D means	1.06	<0.001	36	14.9
65-85 mm				
+/-D	2.33	NS	36	
spring means	1.65	<0.001	36	
1,3-D means	1.04	<0.001	36	35.8
ware yield				
+/-D	3.60	NS	36	
spring means[a]	2.54	<0.001	36	
1,3-D means[b]	1.61	<0.001	36	16.0
total yield				
+/-D	3.52	NS	36	
spring means	2.49	0.002	36	
1,3-D means	1.57	<0.001	36	13.7

[a b]see Table 3.8

Table 3.13. *The effects of fumigation and granular nematicide treatment on the percentage of tubers of ware grade and the mean tuber weight at 121 days after planting*

	% ware			Mean tuber wt.		
	-D	+D	spring means	-D	+D	spring means
untreated	76.1	87.8	82.0	0.0695	0.0985	0.0840
aldicarb	87.3	89.9	88.6	0.0939	0.1084	0.1012
oxamyl	88.3	91.7	90.0	0.1065	0.1198	0.1131
fosthiazate	85.0	89.4	87.2	0.0877	0.1070	0.0974
spring 1,3-D	83.4	88.2	85.8	0.0864	0.0984	0.0924
1,3-D means[b]	84.0	89.4		0.0888	0.1064	

	SED	Significance (P =)	df	CV %
% ware				
+/-D	2.23	0.048	36	
spring means	1.58	<0.001	36	
1,3-D means	1.00	<0.001	36	4.1
Mean tuber wt.				
+/-D	0.00750	NS	36	
spring means	0.00530	<0.001	36	
1,3-D means	0.00335	<0.001	36	12.1

[a b]see Table 3.8

Regression analysis of the total yield of tubers on those of ware grade (Fig. 3.3) shows a significant relationship ($P < 0.001$) with a r^2 value of 0.54. Regression analysis of the total yield of tubers on of the total number of tubers (Fig. 3.4) also showed a significant relationship ($P < 0.001$) with a r^2 value of 0.40. In both cases, the differences between treatments were significant but since there were no interactions and all of the treatments behaved in the same way, the mean for all treatments is shown.

The effect of treatment on the overall yield of tubers and on tuber size distribution is shown in Fig. 3.5. The untreated control had the highest yield of tubers less than 45 mm and the lowest yield of tubers greater 65-85 mm. In the absence of autumn 1,3-D, oxamyl had the lowest yield of tubers less than 45 mm (along with aldicarb) and the highest yield of tubers greater 65-85 mm. In the presence of autumn 1,3-D, oxamyl again had the lowest yield of tubers less than 45 mm and the highest yield of tubers 65-85 mm. The results demonstrate a shift in tuber size distribution with increased yields. The higher yielding treatments have less non-ware grade yield compared to the lower yielding treatments and more ware grade yield. This supports the results in Table 3.13 which show that the highest yielding treatment of autumn 1,3-D and oxamyl had the highest percentage of tubers of ware grade.

Regression analysis of the total yield of tubers on the mean percentage ground cover (Fig. 3.6) showed a significant relationship ($P < 0.001$) with a r^2 value of 0.64. The differences between treatments were again significant but since there were no interactions and all of the treatments behaved in the same way, the mean for all treatments is shown. A correlation between root invasion per g root and total yield was done. This was highly significant ($P < 0.001$) and had an r^2 of 0.34.

Key findings

- yield of non-ware grade tubers (< 45 mm) was decreased by aldicarb and oxamyl
- autumn 1,3-D significantly increased yield of tubers of 45-65 mm and 65-85 mm grades
- all granular nematicides increased yield of tubers of 65-85 mm grade
- autumn 1,3-D and all granular nematicides significantly increased ware and total yields of tubers
- autumn 1,3-D and all granular nematicides significantly increased percentage ware yield
- mean tuber weight increased by autumn application of 1,3-D and all of the granular nematicides

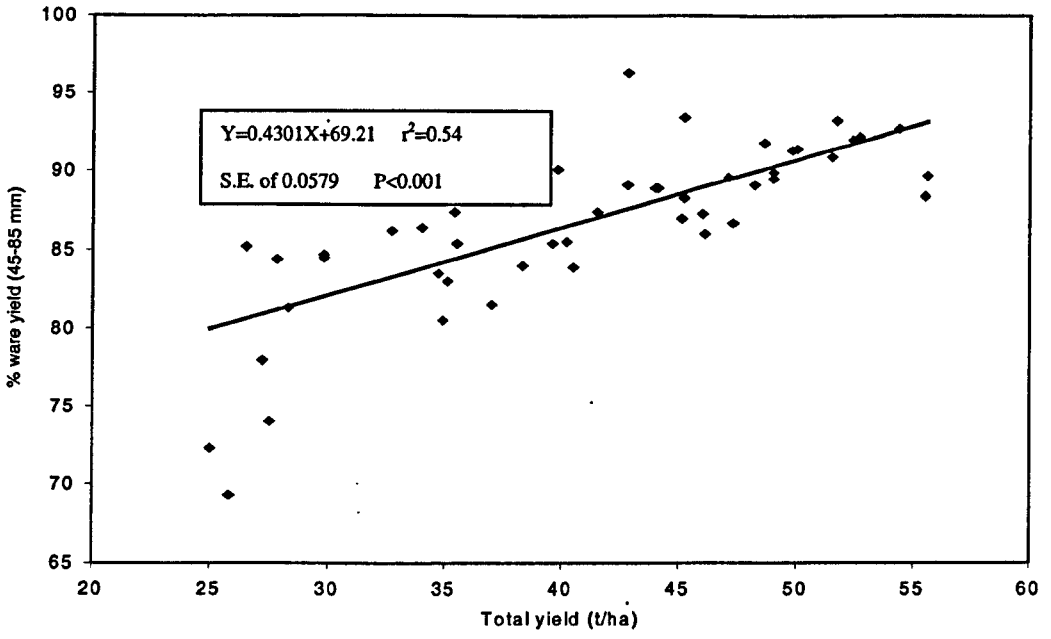

Fig. 3.3. The relationship between the total yield of tubers (t ha^{-1}) and the percentage of tubers of ware grade (45 85 mm)

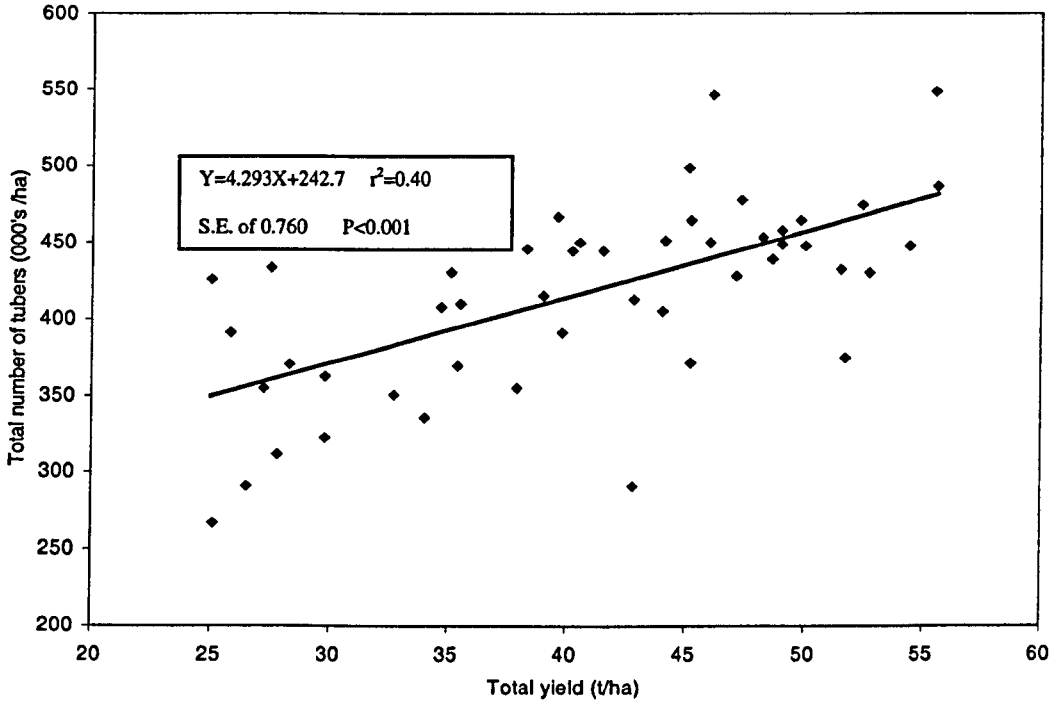

Fig. 3.4. The relationship between the total yield of tubers (t ha^{-1}) and the total number of tubers (000's ha^{-1})

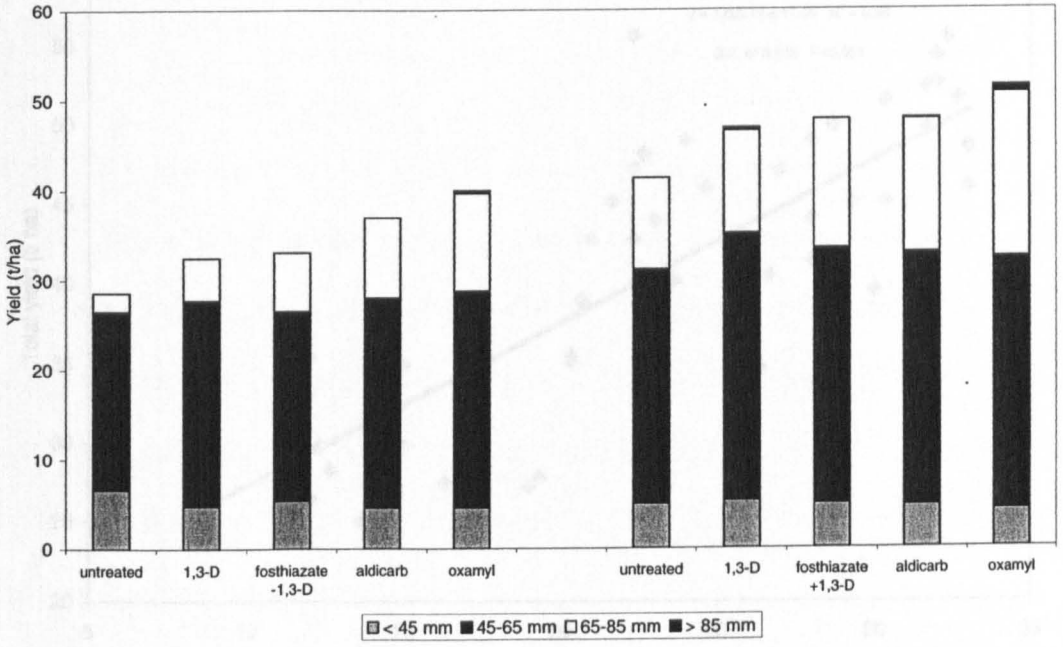

Fig. 3.5. The effects of fumigation and granular nematicide treatment on yield (t ha^{-1}) of individual grades of potato tubers at 121 days after planting

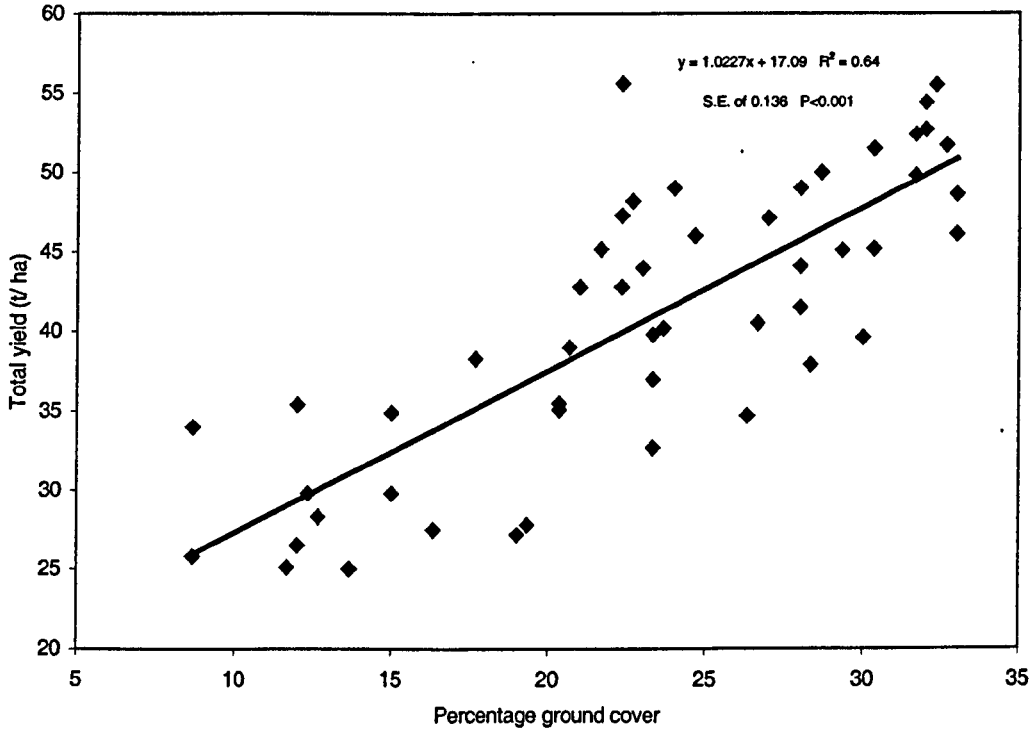

Fig. 3.6. The effects of ground cover on yield (t ha^{-1}) of individual grades of potato tubers at 121 days after planting

3.3.7 Tuber numbers

The effects of fumigation and granular nematicide treatment on number of tubers (000's ha^{-1}) in different size grades at 121 days after planting are shown in Table 3.14. The number of tubers less than 45 mm was significantly decreased ($P = 0.004$) by all of the granular nematicide treatments. The number of tubers 45-65 mm was significantly increased ($P = 0.001$) by the autumn application of 1,3-D. The number of tubers 65-85 mm was significantly increased ($P < 0.001$) by the autumn application of 1,3-D and all spring treatments ($P < 0.001$).

Table 3.15 shows the effects of fumigation and granular nematicide treatment on number of ware grade tubers (000's ha^{-1}), total number of tubers and percentage ware grade tubers at 121 days after planting. The number of ware tubers was significantly increased ($P < 0.001$) by the autumn application of 1,3-D. The total number of tubers was also significantly increased ($P < 0.001$) by the autumn application of 1,3-D. The percentage of ware grade tubers was significantly increased ($P < 0.001$) by the autumn application of 1,3-D and by the three granular nematicides.

The effect of treatment on the overall number of tubers and the number of tubers for individual grades is shown in Fig. 3.7. The results show that the in the absence of 1,3-D, the untreated control had the highest number of tubers less than 45 mm and the lowest number of tubers greater 65-85 mm. Oxamyl had the lowest number of tubers less than 45 mm and the highest number of tubers greater 65-85 mm. In the presence of autumn 1,3-D, oxamyl had the lowest number of tubers less than 45 mm and the highest number of tubers 65-85 mm. Fosthiazate had the highest number of tubers less than 45 mm and the untreated control had the lowest number of tubers 65-85 mm. The results show a shift in the

distribution of the numbers of tubers in individual grades as a result of treatment. This supports the results in Table 3.15 which shows that both 1,3-D and the three granular nematicides increased the percentage of tubers that were of ware grade.

Key findings

- number of non-ware grade tubers (< 45 mm) was decreased by all granular nematicides
- autumn 1,3-D significantly increased the number of tubers of 45-65 mm and 65-85 mm
- all granular nematicides increased the number of tubers 65-85 mm
- autumn 1,3-D significantly increased number of tubers of ware grade and total number of tubers
- the percentage of ware grade tubers was significantly increased by autumn 1,3-D and by the three granular nematicides

Table 3.14. *The effects of fumigation and granular nematicide treatment on number of tubers (000's ha^{-1}) in different size grades at 121 days after planting*

	< 45 mm	45-65 mm	65-85 mm
spring means[a]			
untreated	175.1	215.0	27.5
aldicarb	141.1	226.2	49.9
oxamyl	128.2	219.7	60.5
fosthiazate	141.7	229.1	39.5
spring 1,3-D	161.0	230.9	39.6
1,3-D means[b]			
-D	152.6	208.5	28.8
+D	146.3	239.9	58.0

	SED	Significance (P =)	df	CV %
≤ 45 mm				
+/-D	17.36	NS	36	
spring means[a]	12.27	0.004	36	
1,3-D means[b]	7.76	NS	36	18.4
45-65 mm				
+/-D	20.00	NS	36	
spring means	14.14	NS	36	
1,3-D means	8.95	0.001	36	14.1
65-85 mm				
+/-D	8.19	NS	36	
spring means	5.79	<0.001	36	
1,3-D means	3.66	<0.001	36	29.8

[a b]see Table 3.8

Table 3.15. *The effects of fumigation and granular nematicide treatment on number of ware grade tubers (000's ha^{-1}), total number of tubers (000's ha^{-1}) and percentage ware grade tubers at 121 days after planting*

	no. of ware	total no.	% no. of ware
spring means[a]	242.5	417.6	58.2
untreated	276.3	417.4	66.0
aldicarb	281.1	409.3	69.0
oxamyl	268.5	410.3	65.6
fosthiazate	270.7	431.7	62.5
spring 1,3-D			
1,3-D means[b]			
-D	237.4	389.9	61.4
+D	298.3	444.5	67.2

	SED	Significance (P =)	df	CV %
Ware number				
+/-D	21.83	NS	36	
spring means[a]	15.44	NS	36	
1,3-D means[b]	9.76	<0.001	36	12.9
Total number				
+/-D	29.58	NS	36	
spring means	20.91	NS	36	
1,3-D means	13.23	<0.001	36	11.2
% ware				
+/-D	3.37	NS	36	
spring means	2.38	<0.001	36	
1,3-D means	1.51	<0.001	36	8.3

[a b]see Table 3.8

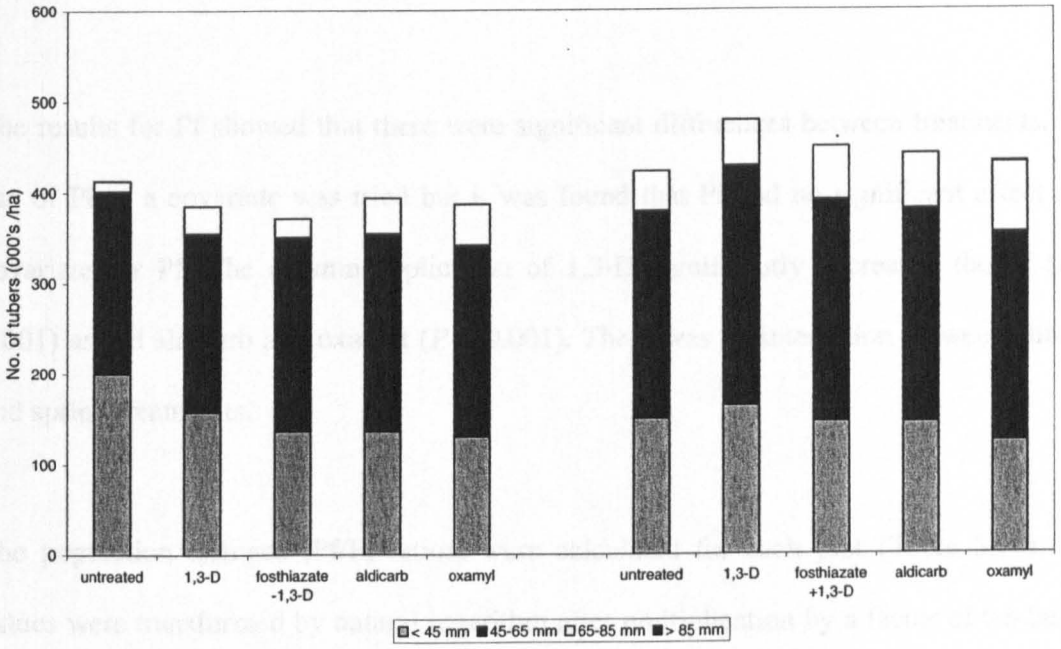

Fig. 3.7. The effects of fumigation and granular nematicide treatment on number of tubers (000's ha^{-1}) in different size grades at 121 days after planting

3.3.8 Nematode multiplication

The initial (Pi) and final (Pf) population densities of PCN (eggs g^{-1} soil) are in Table 3.16. The Pf values were transformed by natural logarithm. The mean Pi for all plots was 103 eggs g^{-1} soil. An analysis of variance for the Pi results show that there were no significant differences between treatments. However, the untreated plots and those which were used for the two fosthiazate treatments did have slightly higher initial densities, although these differences were not significant (Table 3.16).

The results for Pf showed that there were significant differences between treatments. The use of Pi as a covariate was tried but it was found that Pi had no significant effect as a covariate for Pf. The autumn application of 1,3-D significantly decreased the Pf ($P < 0.001$) as did aldicarb and oxamyl ($P = 0.001$). There was no interaction between autumn and spring treatments.

The population changes (Pf/Pi ratios) were calculated for each plot (Table 3.17). The values were transformed by natural logarithm after multiplication by a factor of ten before transformation in order that the final transformed values would be positive (Little & Hills, 1978). It was found that Pi had a significant effect as a covariate for the Pf/Pi ratios ($P = 0.002$). The Pf/Pi ratios were significantly decreased by the autumn application of 1,3-D ($P < 0.001$). The application of aldicarb and oxamyl significantly decreased the Pf/Pi ratio when compared to the untreated control and the spring application of 1,3-D ($P = 0.034$).

Key findings

- no significant differences for Pi between treatments
- autumn 1,3-D, aldicarb and oxamyl decreased the Pf

- autumn 1,3-D significantly decreased the Pf/Pi ratio
- aldicarb and oxamyl significantly decreased the Pf/Pi ratio compared to the untreated control and spring 1,3-D

Table 3.16. *The initial (Pi) and Log$_e$ final (Pf) population densities of potato cyst nematodes (eggs g^{-1} soil) (untransformed data in parenthesis)*

	Pi			Pf		
Treatment	-D	+D	spring means[a]	-D	+D	spring means
untreated	126	131	129	6.101 (455)	5.080 (251)	5.591 (353)
aldicarb	83	81	82	5.281 (212)	4.195 (69)	4.738 (141)
oxamyl	90	106	98	5.582 (278)	4.090 (76)	4.836 (177)
fosthiazate	100	121	111	6.036 (439)	4.924 (153)	5.480 (296)
spring 1,3-D	119	70	94	6.442 (645)	4.829 (142)	5.635 (394)
1,3-D means[b]	104	102		5.888 (406)	4.624 (138)	

Mean Pi for all plots 103

	SED	Significance (P =)	df	CV %
Pi				
+/-D	30.2	NS	36	
spring means[a]	21.4	NS	36	
1,3-D means[b]	13.5	NS	36	46.5
Pf				
+/-D	0.3658	NS	36	
spring means	0.2587	0.001	36	
1,3-D means	0.1636	<0.001	36	11.0

[a b]see Table 3.8

Table 3.17. *The effects of fumigation and granular nematicide on Log_e Pf/Pi (adjusted for Pi as a covariate) (untransformed data in parenthesis)*

Treatment	-D	+D	spring means[a]
untreated	3.826 (4.31)	2.982 (2.15)	3.404 (3.23)
aldicarb	3.113 (3.28)	1.985 (0.85)	2.549 (2.07)
oxamyl	3.485 (4.16)	1.846 (0.77)	2.665 (2.46)
fosthiazate	3.749 (4.56)	2.938 (1.83)	3.194 (3.19)
spring 1,3-D	4.139 (5.76)	2.704 (2.01)	3.421 (3.88)
1,3-D means[b]	3.663 (4.41)	2.431 (1.52)	

	SED	Significance (P =)	df	CV %
Log_e Pf/Pi				
+/-D	0.4048	NS	35	
spring means[a]	0.2862	0.034	35	
1,3-D means[b]	0.1810	<0.001	35	
covariate (Pi)	0.5624[c]	0.002	35	21.0

[a b]see Table 3.8

[c] SE

3.4 Discussion

3.4.1 The effect of 1,3-D on the germination and growth of weed seeds

The numbers of weeds that had germinated and were growing under field conditions were greatly reduced in the soil treated with 1,3-D (Table 3.2). As a result of this, the percentage of ground covered by weeds was decreased by the use of 1,3-D (Table 3.3). This would be expected since where more weeds germinated and grew, the percentage of ground covered by weeds would be significantly increased. The untransformed data indicate a four-fold increase in ground cover in untreated plots and this order of magnitude of difference is comparable to the increase in number of weeds germinating in untreated plots (Table 3.2). However, it remains unclear from these assessments whether 1,3-D was having a direct effect by killing seeds or if the gas in the soil was having a phytotoxic effect on seedlings after they had germinated.

There was a wide range of weed species on the experimental site but mostly annual broad-leaved weeds that have seeds as their means of survival and dispersal. The weed species germinating under glasshouse conditions in the first and second set of samples were similar, although there were a few cases where a species was detected in one set but not the other. All of the weeds were annuals except Corn Mint *(M. arvensis).* The results for the first set of soil samples collected demonstrate a significant decrease in the numbers of weed seeds germinating under glasshouse conditions from treated plots suggesting that the weed seed bank population was decreased by treatment with 1,3-D.

Amost double the number of weed seeds germinated in the first set of samples compared with the second set of samples. The second set of samples was collected after the spring application of 1,3-D which will have killed more seeds, so further decreasing the numbers

germinating. In addition, the second set of samples was collected later in the year and some of the seeds may already have germinated. However, this effect would be small since the cores were taken to a depth of 20 cm and only seeds near the surface would be expected to germinate. As in the previous set of samples, the numbers of Field Pansy *(V. arvensis)* was significantly decreased. The numbers of weeds of other species were also decreased but not significantly.

There were fewer numbers of weeds germinating and growing on potato ridges after treatment with 1,3-D although these differences were not statistically significant. The effects of autumn and spring applications of 1,3-D appeared to be additive since plots treated twice had fewer germinating weeds than plots treated only once. Since the assessments were made 62 days after spring fumigation, any gas would have been dispersed from the soil during the cultivation operations before and at planting. Therefore, the reduced numbers of weeds germinating in treated soil indicate that 1,3-D decreased the numbers of viable seeds in the weed seed bank.

Overall, the results show that 1,3-D has a significant effect in reducing the numbers of germinating weeds in soil after fumigation. Since the results for the germination tests only showed a two-fold decrease in the number of viable seeds germinating, it would therefore appear that the main effect of 1,3-D is a phytotoxic effect on young seedlings in treated soil. The effect on the weed seed bank is less strong since seeds are harder to kill than growing plant tissues. These results agree with the findings of other workers who reported the control of weeds by 1,3-D. For example, Altman & Fitzgerald (1960) found that autumn application of D-D (1,2-dichloropropane, 1,3-dichloropropene) prior to planting reduced weed populations in sugar beet and thought that part of this was a direct effect of

the chemical on the weed seed. Turner, Greathead & Welch (1974) reported control of weeds including Groundsel (*Senecio vulgaris*) by 1,3-D and Coupland & Peabody (1980) suggested that 1,3-D was an effective treatment for the control of field horsetail (*Equisetum arvense*). Bond & White (1984) investigated using 1,3-D followed by either rolling or sheeting with polyethylene for weed control and found that it was highly effective when used at twice the dose used for PCN control.

Herbicides are used in 98.6% of ware potato crops with an average of 1.7 spray applications per crop applying an average of just over three active ingredients to each crop including repeat applications of the same active ingredients (Thomas, Garthwaite & Banham, 1997). Herbicides applications using up to three active ingredients could add up to £100 ha^{-1} to the cost of production based on current prices (Nix, 2000). The experimental site contained a large range of weeds (Table 3.4), some of which were present in large numbers and so in this experiment, a pre-emergence and a post-emergence herbicide were applied. However, the additional control of weeds from the use of 1,3-D may enable a reduction in the number of applications of active ingredients used in field with a less severe weed problem. Since it is possible that 1,3-D may control certain weed species more efficiently than others, then in certain fields the use of 1,3-D could reduce herbicide applications and so increase the profitability of the crop.

3.4.2 Plant emergence

Plant emergence was advanced by 1,3-D, aldicarb and oxamyl (Table 3.8) and it was found that although 1,3-D had a significant effect on emergence over a longer time period that the granular nematicides, the final emergence was unaffected by any of the treatments. This would be expected when growing the cultivar Estima on a site containing PCN at an initial

population density of 103 eggs g^{-1} soil since this level of infestation would be insufficient to cause the plants to be killed before emerging. Therefore, the nematode attack was not killing any of the plants but, since the time of emergence was less advanced for untreated plants, it seems likely that root invasion and subsequent damage of the roots by PCN slowed the overall development of the plant.

This is supported by other published research. Grove, Haydock, Evans & Lewis (1999*a*) found significant improvements in the time taken for plant emergence of both Santé and Pentland Dell following the application of oxamyl. They suggested that the oxamyl at least partially decreased the effects of PCN. The delay in emergence in plots not treated with oxamyl could have arisen from changes in the mineral nutrition of the pre-emerged plants which, although still dependent on the mother tuber for most nutrition, forms roots which increase the supply of mineral ions to the growing plant (Moorby & Milthorpe, 1975). Grove *et al.* (1999*a*) commented that if PCN invade these young roots, a very early reduction in plant nutrient uptake can occur, with an associated reduction in plant growth.

Grove, Haydock, Evans & Lewis (1999*b*) found that plant emergence was significantly more advanced in oxamyl treated plots at 33, 35 and 37 days after planting but by 40 days after planting these differences between treatments disappeared as plots approached maximum emergence. This is a similar pattern to the work reported here (Table 3.8), where it was found that plant emergence was more advanced by all three granular nematicides at 29 days after planting but by 35 days after planting, these differences had disappeared. The increase in plant emergence as a result of the application of 1,3-D was observed at 35 days after planting but had disappeared by 47 days after planting.

3.4.3 Percentage ground cover

The percentage ground cover was significantly increased by both applications of 1,3-D and by all three granular nematicides (Table 3.9). It was found that the effect of 1,3-D on ground cover increased over time. It appears that the nematicides were able to reduce the damage caused by PCN in slowing the rate of leaf expansion and so the leaves would be able to intercept a higher percentage of light which would lead to higher yields. The percentage of ground covered by leaves when viewed from above has been shown to correlate well with percentage light interception (Burstall & Harris, 1983). Regression analysis of yield against ground cover showed an increase in yield with an increase in ground cover (Fig. 3.6) and this is supported by previous work.

Trudgill, Evans & Phillips (1998) found that light interception is dependent initially on the rates of expansion of haulms of individual plants. In healthy crops, the canopies of individual plants meet within, and then between, rows. PCN slows the rate of haulm expansion and so increases the time taken for the leaves of individual plants to merge. This decreases the efficiency of light interception (Trudgill, Evans & Parrott, 1975*a,b*). The decreased top growth in the early part of the season and the premature senescence in the late part of the season account for much of the reduction in yield (Trudgill, 1986). This is also supported by Haverkort & Trudgill (1995) who noted that PCN decreased potato tuber yields by decreasing rates of top growth (thereby increasing the time taken from crop emergence to 100% light interception), by increasing the rate of senescence, by decreasing photosynthetic and water-use efficiency, and by changes in dry-matter composition.

3.4.4 Root invasion

Juvenile PCN hatch in response to specific factors in potato-root exudates and are attracted to growing roots by factors in root exudates (Trudgill *et al.*, 1998). Lateral roots are particularly vulnerable to stunting as a consequence of root invasion and damage and reduced root growth can be detected within a few days of attack. The tops of heavily infested plants are stunted early in their growth (Trudgill *et al.*, 1998).

The invasion of plant roots by juveniles was significantly decreased by the autumn application of 1,3-D (Table 3.10) which suggests that 1,3-D killed significant numbers of juveniles within the cysts which were therefore unable to invade the plants. Root invasion was lowest for aldicarb and oxamyl in combination with 1,3-D (110 juveniles g^{-1} root) while the highest was for the untreated plots (2150 juveniles g^{-1} root). Clearly, the huge differences seen in invasion and the subsequent differences in the amount of damage caused will account for the differences in yield that were observed. A correlation between root invasion per g root and total yield was done and was found to be highly significant ($P < 0.001$) with a r^2 of 0.34. Evans, Trudgill & Brown (1977) and Trudgill *et al.* (1975*a,b*) found that root invasion per g root is related to crop yield and as invasion increases, the yield decreases in the majority of cases.

The three granular nematicides significantly decreased root invasion compared to the untreated control, suggesting that they were paralysing the juveniles in the soil and preventing them reaching the roots. Woods, Haydock & Edmunds (1999) reported that organophosphate and oxime carbamate nematicides applied at their respective field rates do not kill nematodes directly in the soil but their principal mode of action is nematostasis. They paralyse nematodes to such an extent that they are impaired in their ability to locate a

suitable host plant (Hague & Pain, 1970; Nelmes, 1970; Wright & Womack, 1981). Woods *et al.* (1999) reported that nematicides may cause fewer juveniles to hatch. This has been reported for aldicarb (Osborne, 1973) and oxamyl (Evans & Wright, 1982). Woods *et al.* (1999) concluded that it is likely that the two mechanisms are additive with suppression of hatch being the initial mechanism followed by paralysis of juveniles after hatch has occurred.

Aldicarb and oxamyl were more effective than fosthiazate at reducing root invasion. It was surprising that fosthiazate was less effective than the other two nematicides in this experiment since Woods & Haydock (2000) found that fosthiazate effectively reduced root invasion when incorporated to the correct depth on a sandy clay loam soil in a field experiment in Shropshire. The experimental site here reported here was a loamy sand and it is possible that the poor performance of fosthiazate in regard to root invasion was due to differences in the soil type.

Root invasion was not significantly different between the spring application of 1,3-D and the untreated control, suggesting that the spring application of 1,3-D had not been effective reducing the initial nematode population. The reasons for this remain unclear.

3.4.5 Incidence of *R. solani*

The results show that there was a lower incidence of disease on plants that were in plots treated with 1,3-D although these differences were not significantly different. Since Altman & Fitzgerald (1960) had previously found that 1,3-D had reduced *Rhizoctonia* infection, it was decided to make further assessments during the next field experiment (Chapter 4).

3.4.6 Tuber yield

The spring and autumn application of 1,3-D and all three granular nematicides significantly increased ware yields, total yields and percentage ware yields. The range in the total yields was large, with the lowest yields in untreated plots (28.6 t ha^{-1}) and the highest in plots treated with 1,3-D and oxamyl (51.4 t ha^{-1}). Treatment with oxamyl alone increased yield to 40.1 t ha^{-1} and the autumn application of 1,3-D increased yields to 41.3 t ha^{-1}.

The differences in yield between treatment found here are very similar to those reported by Barker *et al.* (1998). On a site containing *G. pallida* at a lower population density of 30 eggs g^{-1} soil and with the cultivar Estima, they found that the control treatment had a total yield of 29.7 t ha^{-1} and plots treated with 1,3-D and oxamyl had a yield of 53.6 t ha^{-1}. Plots treated with oxamyl alone had a yield of 37.0 t ha^{-1} and the application of 1,3-D gave a yield of 41.9 t ha^{-1}. In both of these experiments, treatment with either 1,3-D or oxamyl increased yield, with a better benefit from 1,3-D than oxamyl and an additive effect for the combination of both treatments. This is further supported by Whitehead & Nichols (1992*a*) who reported that plots treated with 1,3-D followed by a spring application of a granular nematicide (aldicarb or oxamyl) had higher yields than those treated with only the granular nematicide. Whitehead *et al.* (1994) also reported that 1,3-D increased tuber yields more than oxamyl.

Previous work has shown that the increase in yield after application of 1,3-D is at least partly due to its effects on nitrogen mineralisation. Martin & Pratt (1958) noted that nitrifiers appeared to be more sensitive to soil fumigants than ammonifiers and this reduces

their activity. In contrast, the organisms that release ammonium nitrogen increase in numbers quickly following treatment leading to a rapid accumulation of ammonium nitrogen in the soil. Other studies have indicated that soil fumigants retard or inhibit nitrification of ammonium nitrogen (Wolcott *et al.*, 1967; Jenkinson & Powlson, 1970). Elliot *et al.*, (1974) found increased levels of NH_4^+-N and reduced levels of NO_3^--N in soils that had been fumigated. The NH_4^+-N in the soil is leached less rapidly and cannot be lost by denitrification (Rovira, 1976). Williams & Salt (1970) also found that mineralised nitrogen increased after treatment with D-D (1,2-dichloropropane, 1,3-dichloropropene mixture). Rovira (1976) found an increase in nitrogen uptake in wheat after fumigation with chloropicrin and concluded that this was partly due to an enhancement of ammonium release and inhibition of nitrification with a consequent increase in uptake by the plants. Tu (1996) found that Telone C (1,3-dichloropropene and related C_3 hydrocarbons - 85%; chloropicrin - 15%) decreased nitrification activity. Therefore, the nitrogen mineralisation effect of 1,3-D appears to be a major factor in its ability to increase yields.

Although the differences in yield were similar between treatments for the work reported here and for the work by Barker *et al.* (1998), there was considerable variation in the initial nematode population densities (103 and 30 eggs g^{-1} soil respectively). Trudgill (1986) reviewed the relationship between yield losses caused by PCN and the increase in yields resulting from nematicide treatment and stated that there is considerable variation between sites in the damage caused by PCN. At low population densities, the variation appears to be less. Moss, Crump & Whitehead (1975) found no significant differences in yields between treated and untreated plots at lightly infested sites (Pi of less than 13 eggs g^{-1} soil).

Trudgill *et al.* (1983) reported that oxamyl had no effect on yields of six potato cultivars at a PCN-free site. Trudgill (1986) found that at sites infested with 25-30 eggs g^{-1} soil, the yield increase varied from 3-28 t ha^{-1} but at a heavily infested site with 661 eggs g^{-1} soil, the yield increase was only 13.5 t ha^{-1}. Some of the variation between sites is probably due to differences in the efficiency of nematicide application and of measuring nematode density or yield but the differences are so great that other factors must be involved. Overall, the results suggest that yield losses caused by PCN, and the corresponding increase resulting from applying nematicide, may differ greatly between sites with similar nematode densities.

The growth and yield of potato plants is depressed when they are infected by PCN, and for individual cultivars, a direct relationship between the pre-planting nematode population density and yield can be demonstrated (Evans & Haydock, 1990). The relationship between nematode density at planting and yield has led many people to try to develop models to describe it. Brown (1969) made observations on yield of potatoes attacked by PCN. From these observations, he was able to make the generalisation (based on observations from a range of cultivars with different tolerance levels) that yield loss would be 2.13 t ha^{-1} for each 20 eggs g^{-1} soil of the nematode. Brown (1983) revised this rate of loss upwards to 6.2 t ha^{-1} for each 20 eggs g^{-1} soil. Whitehead *et al.* (1984) found that *G. rostochiensis* decreased the yield of Désirée potatoes by 8.2 t ha^{-1} for every increment of 20 eggs g^{-1} soil.

The results from this experiment show a yield of 28.6 t ha^{-1} for the control at an initial population density of 100 eggs g^{-1} soil. Using Brown's (1983) prediction of yield loss, it would be expected that the yield on uninfested land would be about 59.6 t ha^{-1}. Many

workers have found that nematicides do not give complete protection from PCN damage. At sites moderately or heavily infested with PCN, trials have shown that damage is not always wholly prevented by treatments with aldicarb or oxamyl at the recommended rates which are a compromise between cost and effectiveness (Trudgill, 1986). It has been found by several workers that applying more than the recommended rate can be increased especially for intolerant cultivars (Whitehead *et al.*, 1973a; Moss *et al.*, 1975; Trudgill, Mathias & Tones, 1985). The highest yielding treatment of autumn 1,3-D and oxamyl yielded 51.4 t ha^{-1}. If it was accepted that at this high population density, the nematicides do not give complete protection from PCN damage, then it may have reached up to 60 t ha^{-1}. Therefore in this experiment, Estima is showing the same yield losses as predicted by Brown (1983) for a range of cultivars.

It was found that the differences in yield were related to the ground cover of plants (Fig 3.6) and that the mean plant emergence was found to be significantly correlated to the mean ground cover ($P < 0.001$) with a r^2 value of 0.53. The use of 1,3-D and the granular nematicides advanced the time of emergence and the ground cover of plants which would lead to increases in yield (Trudgill, 1986). A correlation between root invasion and yield was highly significant ($P < 0.001$) and had an r^2 of 0.34 indicated that increased root invasion had led to more damage of the roots and therefore less yield. Treatment by 1,3-D and the granular nematicides advanced the emergence and ground cover of plants and reduced root invasion and this will have contribute to the increases in yield observed.

3.4.7 Tuber numbers

The total number of tubers and the number of tubers of ware grade were not significantly increased by any of the spring treatments but the percentage of tubers of ware grade was

increased by all of the spring treatments, indicating that there had been a shift in the tuber size distribution (Table 3.15). The results for the individual ware grades show that there were more smaller tubers (less than 45 mm) and less larger tubers (greater than 45 mm) in those plots that were untreated in the spring. So although the total numbers of tubers were unaffected, their average size was greater. This is supported by the yield results (Table 3.13) which shows that the spring treatments significantly increased the mean tuber weight.

The total number of tubers was significantly increased by the autumn application of 1,3-D (Table 3.15) which is in contrast to the results of Barker *et al.* (1998) who found that 1,3-D reduced tuber numbers. There is little published work on the effect of nematicides on tuber number and the reasons for these different effects remains unclear and should be investigated further. Results for the individual ware grades show that the tuber size distribution was again affected. In this case, although the number of smaller tubers (less than 45 mm) was not significantly decreased, there were more larger tubers (greater than 45 mm) in those plots that were treated. The effect of 1,3-D was therefore similar to that of the granular nematicides but greater in that the number of tubers was increased. Barker *et al.* (1998) also found that 1,3-D improved tuber size distribution.

3.4.8 Nematode multiplication

The experiment had a high initial population density (103 eggs g^{-1} soil) which was at a level at which the use of a combination of fumigation and granular nematicides would be considered in commercial practice. This level of infestation made the site suitable for an experiment. An analysis of variance for the Pi results showed that there were no significant differences between treatments. Although the untreated plots and those which were used for the two fosthiazate treatments did have slightly higher initial densities, these

differences were not statistically significant (Table 3.16). However, it was found that Pi had a significant effect as a covariate for the Pf/Pi ratios. This would be expected since it has already been established that PCN multiplication rates are density dependent (Turner & Evans, 1998; Phillips & Trudgill, 1998).

There was a nearly ten-fold difference in the range in Pf values, depending on treatment. The combination of 1,3-D and aldicarb had the lowest Pf value (69 eggs g^{-1} soil) while the spring application of 1,3-D had the highest Pf value (645 eggs g^{-1} soil) which was much higher than the untreated control (455 eggs g^{-1} soil). Clearly, 1,3-D on its own did not decrease nematode multiplication. The use of two applications of 1,3-D had a Pf of 142 eggs g^{-1} soil, which is much more acceptable. The Pf and Pf/Pi values were significantly decreased by aldicarb and oxamyl but not fosthiazate, indicating that, in this experiment, fosthiazate was less effective at controlling nematode multiplication. This again is in contrast to Woods & Haydock (2000) who found that fosthiazate controlled nematode multiplication effectively when incorporated to the correct depth. In this experiment, all nematicides were incorporated to the same depth and so the reason why fosthiazate performed less well may be due to soil type. An example of this is the organophosphorus nematicide ethoprophos (Mocap) which may have reduced efficacy on organic soils (Whitehead, 1999).

There were large differences in the Pf/Pi ratios between treatments. The lowest Pf/Pi ratios were for the combinations of 1,3-D and oxamyl (0.77) and 1,3-D and aldicarb (0.85) while the highest was for the spring application of 1,3-D (5.76) with the untreated control having a ratio of 4.31. Since a ratio of less than one indicates that there has been a reduction in the number of nematodes, aldicarb and oxamyl in combination with 1,3-D were seen to reduce

the nematode populations when compared to the untreated control. The analysis showed that 1,3-D significantly decreased the untransformed ratio, from 4.41 to 1.52.

The control of PCN multiplication by the use of nematicides was previously studied by Whitehead, Nichols & Senior (1991) who reported that in soil moderately or heavily infested with *G. pallida*, that oxamyl frequently lessened the Pf/Pi ratios at some sites but not at others. Whitehead & Nichols (1992*b*) found that oxamyl decreased numbers of *G. rostochiensis* eggs in the soil when the cultivar Désirée was grown but not after the resistant cultivar Cara was grown.

Whitehead *et al.* (1994) found that in plots of Désirée treated with 1,3-D and the granular nematicide ethoprophos, nematode multiplication was controlled better than in plots treated with ethoprophos alone. In plots treated with 1,3-D and fosthiazate, there was less multiplication by *G. pallida* on Désirée in one trial but not in another. Whitehead & Nichols (1992*a*) found that the Pf/Pi ratio was decreased in plots treated with aldicarb or oxamyl alone compared with plots treated with 1,3-D plus aldicarb or oxamyl. Whitehead *et al.* (1994) concluded that 1,3-D did not lessen *G. pallida* multiplication. The application of 1,3-D effectively reduces the initial population density of the nematode population. The multiplication of PCN is density dependent with greater multiplication at lower population densities (Turner & Evans, 1998). Therefore, by reducing initial population density, 1,3-D may increase the rate of PCN multiplication and may give a higher final population density than if the fumigant had not been used (Phillips & Trudgill, 1998). However, the use of a granule nematicide as used here would control nematode multiplication. Phillips & Trudgill (1998) commented that nematicides are more effective at preventing further increase in small populations of PCN than they are at decreasing large ones.

3.4.9 Economic benefits from nematicide use

An analysis of the economic benefits of the use of the granular and fumigant nematicides, both singly and in combination was undertaken. Values for the variable costs and price of potatoes were obtained from the Farm Management Pocketbook (Nix, 2000) where the five-year average price for maincrop tubers was given as £131 per tonne. The costs of fumigant and nematicide costs were the current commercial costs obtained from chemical distributors (Hodges & Moss, Newport, Shropshire for granular nematicides; Sands Agricultural Services, Holbeach, Lincolnshire for fumigant).

The results (Table 3.18) show that in the absence of autumn 1,3-D, the use of any of the spring treatments increased the gross margin compared to the untreated control. Fosthiazate and the spring application of 1,3-D increased the gross margin by less than £400, while the use of aldicarb had a greater effect (£1039) and the use of oxamyl the greatest effect (£1416). In the presence of autumn 1,3-D, the lowest gross margin was for the control. Fosthiazate and the spring application of 1,3- D increased the gross margin least and oxamyl again gave the greatest increase (£1076).

Therefore, the results show that the gross margins were increased by all of the spring treatments and the autumn fumigation. Autumn application with 1,3-D in combination with the spring treatments further increased the gross margins compared to any of the spring treatments used alone. The highest gross margin (£3252) for the use of autumn 1,3-D and oxamyl was an increase of £2383 compared to the untreated control. The results show that there is a clear economic benefit in the combined use of both an autumn application of 1,3-D and a granular nematicide.

Table 3.18. The gross margins for each of the ten treatments

1,3-D	Spring trt[a]	Variable costs (£ ha^{-1})	Fumigant cost (£ ha^{-1})	Granules cost (£ ha^{-1})	Total variable cost (£ ha^{-1})	Ware yield (t ha^{-1})	Price (£ t^{-1})	Output (£ ha^{-1})	Gross margin (£ha^{-1})
-	untreated	2000	0	0	2000	21.9	131	2868.9	869
-	aldicarb	2000	0	336	2336	32.4	131	4244.4	1908
-	oxamyl	2000	0	352	2352	35.4	131	4637.4	2285
-	fosthiazate	2000	0	363	2363	27.6	131	3615.6	1253
-	1,3-D	2000	0	579	2579	27.7	131	3628.7	1050
+	untreated	2000	579	0	2579	36.3	131	4755.3	2176
+	aldicarb	2000	579	336	2915	43.1	131	5646.1	2731
+	oxamyl	2000	579	352	2931	47.2	131	6183.2	3252
+	fosthiazate	2000	579	363	2942	42.8	131	5606.8	2665
+	1,3-D	2000	579	579	3158	41.5	131	5436.5	2278

3.4.10 Conclusions

The aims of the experiment were to assess the effectiveness of the use of 1,3-D in combination with three granular nematicides for the control of PCN and for an increase in yield. The first objective to be determined was whether 1,3-D in combination with a granular nematicide would control PCN multiplication more than either treatment used singly. It was found that the lowest Pf/Pi ratios were obtained from the combination of 1,3-D with aldicarb (Pf/Pi of 0.85) and 1,3-D with oxamyl (Pf/Pi of 0.77). Since the values are less than one, they represent a decrease in the number of nematodes. The use of 1,3-D without the use of a granular nematicide was higher (Pf/Pi of 2.15) and the use of aldicarb used singly was even higher (Pf/Pi of 3.28) and this was also true for oxamyl used singly (Pf/Pi of 4.16). Therefore, 1,3-D in combination with a granular nematicide controlled PCN multiplication more than either treatment used singly.

The second objective to be determined was whether 1,3-D in combination with a granular nematicide would increase yields more than either treatment used singly. It was found that the highest yielding treatments were the combination of 1,3-D with oxamyl (47.2 t ha^{-1}) and 1,3-D with aldicarb (43.1 t ha^{-1}). The use of aldicarb singly gave a yield of 32.4 t ha^{-1} and the use of oxamyl singly gave 35.4 t ha^{-1}. Plots that were untreated yielded only 21.9 t ha^{-1}. Since there was no interaction between the use of fumigation and granular nematicides, the effects on yield were additive. Therefore, the use of granular nematicides significantly increased yields and the additional use of 1,3-D reduced yield loss even further.

The final objective to be determined was whether 1,3-D in combination with a granular nematicide would reduce plant root invasion more than either treatment used singly. The

results showed that the lowest root invasions were obtained in plants treated with the combination of 1,3-D with aldicarb (transformed value of 4.45 juveniles g^{-1} root) and 1,3-D with oxamyl (transformed value of 4.41 juveniles g^{-1} root). The use of 1,3-D without the use of a granular nematicide had higher invasion (transformed value of 6.20 juveniles g^{-1} root) and the use of aldicarb used singly was higher (transformed value of 5.62 juveniles g^{-1} root) as was oxamyl used singly (transformed value of 5.48 juveniles g^{-1} root). The untreated plants had a transformed value of 7.54 juveniles g^{-1} root. The analysis showed that there was no interaction between the use of 1,3-D and the granular nematicides and that the effects on invasion were additive. Therefore, the use of 1,3-D in combination with a granular nematicide reduced plant root invasion more than either treatment used singly.

4.0 Chapter 4.

Field Experiment Two

The use of the soil fumigant 1,3-dichloropropene in combination with the resistant cultivar Santé and the granular nematicide oxamyl at full and half-rates for the control of potato cyst nematodes

4.1 Introduction

The second field experiment done in 1999 was designed to follow on from the work done in the first field experiment and to take the work a stage further. This experiment would assess the use of 1,3-D with a resistant cultivar and a granular nematicide at full and half-rates. The site chosen contained both species of PCN (mainly *G. rostochiensis*) at nearly 200 eggs g^{-1} soil. This population density is too high to grow potatoes using only a granular nematicide; the use of a soil fumigant would be necessary to bring this land back into production. 1,3-D was injected in the spring due to poor soil conditions during autumn. The cultivars used were Estima (for continuity with the first experiment) and the resistant cultivar Santé. The nematicide oxamyl was chosen since it gave the best performance in the previous experiment.

4.1.1 Aims

The aims of the experiment were to assess the various treatment combinations for the control of nematode multiplication and an increase in yield. The same assessments were made as for the previous experiment and, in addition, growth analysis was performed on the plants after 6 weeks. This permitted the effects of the treatments on plant health to be monitored during the growing season.

The objectives to be determined were:

- whether a resistant cultivar was more effective than a combination of 1,3-D and a granular nematicide for the control of PCN multiplication and for increasing yield
- whether the use of full-rate oxamyl was more effective than half-rates for control of PCN multiplication and for increasing yield

- whether the combined use of a resistant cultivar, 1,3-D and a granular nematicide was the most effective strategy for the control of PCN multiplication and for increasing yield

4.2 Materials and methods

The materials and methods for Field Experiment Two are given below. Since they were similar to those for Field Experiment One, reference is made to relevant sections that fully described the methods in Chapter 3 to avoid repetition.

4.2.1 Experimental design

Th experiment had a two by two by three factorial design. This gave a total of twelve treatment combinations as shown in Table 4.1. There were two cultivars, two levels for fumigation, and three levels for the granular nematicide treatment. The levels for fumigation were either treated or untreated. The three levels of the granular nematicide were zero, half and full-rates of the recommended application rates from the UK Pesticide Guide (Whitehead, 1999).

Table 4.1. *List of treatments for Field Experiment Two*

Treatment	Cultivar	1,3-D rate	Oxamyl rate
1	Estima	untreated	untreated
2	Estima	untreated	2.75 kg a.i. ha^{-1}
3	Estima	untreated	5.5 kg a.i. ha^{-1}
4	Estima	211.5 l a.i. ha^{-1}	untreated
5	Estima	211.5 l a.i. ha^{-1}	2.75 kg a.i. ha^{-1}
6	Estima	211.5 l a.i. ha^{-1}	5.5 kg a.i. ha^{-1}
7	Santé	untreated	untreated
8	Santé	untreated	2.75 kg a.i. ha^{-1}
9	Santé	untreated	5.5 kg a.i. ha^{-1}
10	Santé	211.5 l a.i. ha^{-1}	untreated
11	Santé	211.5 l a.i. ha^{-1}	2.75 kg a.i. ha^{-1}
12	Santé	211.5 l a.i. ha^{-1}	5.5 kg a.i. ha^{-1}

The plot layout for Field Experiment Two is shown in Fig. 4.1. The plot number is indicated on the top row of each square. A double line indicates a tramline. The plots were three beds wide (5.5 m) and nine metres long. Since one bed is made up from two rows of potatoes, so each plot was six rows wide. The middle two rows in each plot were used as the harvest rows. The twelve treatment combinations were replicated in each of the five blocks. The blocks were arranged across the width of the experiment as shown in Fig. 4.1. Blocks 1, 3 and 5 are shaded in grey and blocks 2 and 4 are unshaded.

Blocks

10 *Estima* untreated + ½ oxamyl	**20** *Santé* untreated + oxamyl	**30** *Estima* 1,3-D + untreated	**40** *Santé* 1,3-D + oxamyl	**50** *Estima* 1,3-D + oxamyl	**60** *Estima* 1,3-D + ½ oxamyl
9 *Santé* 1,3-D + ½ oxamyl	**19** *Estima* untreated + untreated	**29** *Estima* untreated + oxamyl	**39** *Santé* 1,3-D + untreated	**49** *Santé* untreated + ½ oxamyl	**59** *Santé* untreated + untreated
8 *Santé* 1,3-D + oxamyl	**18** *Estima* untreated + oxamyl	**28** *Santé* untreated + untreated	**38** *Santé* untreated + oxamyl	**48** *Estima* 1,3-D + untreated	**58** *Santé* 1,3-D + untreated
7 *Estima* untreated + untreated	**17** *Santé* untreated + ½ oxamyl	**27** *Estima* 1,3-D + ½ oxamyl	**37** *Santé* 1,3-D + ½ oxamyl	**47** *Estima* untreated + ½ oxamyl	**57** *Estima* 1,3-D + oxamyl
6 *Estima* 1,3-D + ½ oxamyl	**16** *Santé* untreated + oxamyl	**26** *Estima* untreated + ½ oxamyl	**36** *Estima* 1,3-D + untreated	**46** *Santé* untreated + untreated	**56** *Estima* untreated + untreated
5 *Estima* untreated + oxamyl	**15** *Santé* 1,3-D + oxamyl	**25** *Santé* 1,3-D + untreated	**35** *Estima* 1,3-D + oxamyl	**45** *Santé* 1,3-D + ½ oxamyl	**55** *Santé* untreated + ½ oxamyl
4 *Estima* 1,3-D + oxamyl	**14** *Estima* 1,3-D + untreated	**24** *Estima* untreated + untreated	**34** *Estima* untreated + oxamyl	**44** *Santé* untreated + oxamyl	**54** *Santé* 1,3-D + oxamyl
3 *Santé* untreated + untreated	**13** *Santé* 1,3-D + untreated	**23** *Santé* 1,3-D + ½ oxamyl	**33** *Santé* untreated + ½ oxamyl	**43** *Estima* 1,3-D + ½ oxamyl	**53** *Estima* untreated + ½ oxamyl
2 *Estima* 1,3-D + untreated	**12** *Santé* untreated + untreated	**22** *Santé* untreated + ½ oxamyl	**32** *Estima* 1,3-D + oxamyl	**42** *Estima* untreated + untreated	**52** *Santé* 1,3-D + ½ oxamyl
1 *Santé* 1,3-D + untreated	**11** *Santé* 1,3-D + oxamyl	**21** *Estima* untreated + ½ oxamyl	**31** *Estima* 1,3-D + ½ oxamyl	**41** *Estima* untreated + oxamyl	**51** *Santé* untreated + oxamyl

1
2
3
4
5

9 m

(3 beds, 5.5 m)

Fig. 4.1. Plot layout for Field Experiment Two: The use of 1,3-D in combination with the resistant cultivar Santé and the granular nematicide oxamyl at full and half-rates for the control of potato cyst nematodes.

4.2.2 Selection of experimental site

A site was chosen for the experiment in a field called Four Gates, which is part of Harper Adams University College farm. The topsoil is a slightly stony sandy loam and the subsoil is a moderately permeable clay loam (Beard, 1988). The site had ADAS nutrient indices (Anon, 1994) of P = 5, K = 8, Mg = 3, and a pH of 7.9. The site had been used for a potato experiment the previous year when the cultivar Estima was grown. The site was infested with PCN and contained both species of nematode but mainly *G. rostochiensis.* The site was initially sampled in blocks of 20 m width to determine if it was suitable for use as an experimental site. The results showed a uniform nematode population density across the site when measured for all of the plots, with the average population density being 190 eggs g^{-1} soil.

4.2.3 Sampling soil for PCN

The plots were marked out for the experiment on 31 March 1998. The Pi samples for each plot were taken on the 31 March 1999 and the Pf samples were taken after harvest on the 10 September 1999. Samples were taken as described in 3.2.3.

4.2.4 Cyst detection and estimation of cyst contents

Cysts were detected and cyst contents estimated as described in 3.2.4.

4.2.5 Species identification

Cysts were identified by IEF (Fleming & Marks, 1983) using a 50 cyst sample, and by using the polymerase chain reaction (PCR) (Mulholland *et al.*, 1996; Bulman & Marshall, 1997).

4.2.6 Application of fumigant

After the harvest of the previous potato crop, the site was shakerated in two directions at right angles to one another on the 26 March 1999. The site was ploughed on the 31 March 1999 to prepare soil for the application of 1,3-D. The injection of 1,3-D (as Telone II: 94% a.i. w/w; Dow Agrosciences) at 211.5 litres a.i. ha^{-1} was done using a Rumpstadt Combiject (Rumpstadt, Haringvliet, The Netherlands) on the 1 April 1999. The soil temperature was 9.5°C at 20 cm depth. The width treated by the machine was 3 m so two passes were made to treat each plot. As in the previous experiment, the plots were 5.5 m wide so the area treated was 0.5 m wider than the plots. In this experiment each plot was again beside a tramline (Fig. 4.1), and the excess area treated was able to be part of a tramline.

4.2.7 Application of granular nematicide

After application of 1,3-D, the site was left for 26 days and then was cultivated by ploughing to release any remaining gas in the soil before forming the beds. The site was ridged up to form the beds on the 27 April 1999. On the 30 April, the site was bed-tilled and stone-separated. The granular nematicide oxamyl was applied to the beds using a land-wheel-metered granule distributor on the day of planting (1 May 1999). Oxamyl (as Vydate 10G; 10% a.i. w/w; DuPont) was applied as granules at two rates: 2.75 kg a.i ha^{-1} and 5.5 kg a.i. ha^{-1}. The nematicide was incorporated using a tractor-mounted rotavator to a depth of 15 cm.

4.2.8 Crop management

After soil analysis, fertiliser was applied as required and the crop was managed as according to standard agrochemical practices for the control of weeds and diseases (Appendix 9).

4.2.9 Cultivar planted

The cultivars chosen for the experiment were Estima and Santé. Estima was chosen because it is widely grown commercially, has no resistance to either species of PCN and provided continuity with the previous experiment. The NIAB recommended variety list (Anon, 2000) describes Estima as a high yielding second early which has early bulking and mature yields comparable to early maincrop varieties. It has smooth skin texture, shallow eyes and attractive uniform tubers. It is susceptible to virus Y, powdery scab and blackleg.

Santé is one of the few commercially grown cultivars with partial resistance to *G. pallida* as well as being fully resistant to *G. rostochiensis*. The NIAB recommended variety list (Anon, 2000) describes Santé as a maincrop cultivar which has good yields of round-oval tubers that are slightly flattened. Skin texture is very smooth. It is moderately susceptible to blackleg and gangrene, but has very good virus resistance. It is susceptible to drought stress and has high resistance to bruising.

The NIAB recommended variety list (Anon, 2000) rates both Estima and Santé as 7 for yield (on a scale of 1-9 where 9 = high marketable yield). For tuber number, Estima is rated as 6 and Santé as 7 (on a scale of 1-9 where 9 = high tuber number per plant). For tuber size, Estima is rated as 7 and Santé as 6 (on a scale of 1-9 where 9 = large tubers). Overall, the main difference between the two cultivars is in their resistance to PCN. The

NIAB recommendations rate Santé as moderately resistant to *G. pallida* while Estima is susceptible to both species of PCN. Estima (Super Elite 1, size grade: 45-50 mm) and Santé (Super Elite 1, size grade: 45-50 mm) were planted on 1 May at 15-20 cm depth and 30 cm spacing using a tractor mounted potato planter.

4.2.10 Plant emergence

Plant emergence was measured at 27, 34, 38, 41 and 54 days after planting. The numbers of plants emerged from the middle two rows in each plot were counted as described in 3.2.12.

4.2.11 Percentage ground cover

The percentage of ground covered by the growing potato plants was measured at 38, 47 and 54 days after planting using the same grid as described in 3.2.13 (Burstall & Harris, 1983). In the first field experiment, two readings were taken for each of the two rows in the middle bed; in the second field experiment, this was increased to three readings per row in an attempt to reduce the CV's for the experiment (compare 3.3.3 with 4.3.2). The 3 readings were taken from each of the 2 rows in the middle bed. The first reading was taken approximately 2 metres into the plot and subsequent readings were taken 1 metre apart.

4.2.12 Root invasions

Two plants were taken from each plot at six weeks after planting to measure root invasion as described in 3.2.14.

4.2.13 Growth analysis

Plant samples were taken for growth analysis at 44 days after planting. One plant was taken from each of the two outer rows of the non-harvest beds. Each plant was systematically selected by choosing the 12th plant from the end of the plot. The plants were divided into their component parts and weights were recorded for tops, stems, roots, stolons, and tubers. The numbers of stems and tubers were counted and the total plant weight was calculated from the addition of the weights of the individual parts. The shoot weight /root weight ratio was calculated by dividing the fresh shoot weight of the plant by the fresh root weight excluding the weights of stolons and tubers.

4.2.14 Assessment of incidence of *R. solani*

The plants assessed for *R. solani* were the plants that were used for growth analysis at 44 days after planting as described in 4.2.13 The assessment of *R. solani* incidence was made using a key described by Simons & Gilligan (1997) as described in 3.2.15.

4.2.15 Harvesting and grading

The experiment was desiccated by diquat at 116 days after planting (as Reglone: 200 g l^{-1}, Zeneca) and harvested on the 9 September 1999 (131 days after planting). The ends of the plots were harvested by hand using a fork and a 5 m length was harvested from the two middle rows in each plot using a tractor mounted, two-row potato spinner. The mechanically harvested plots were then hand forked to ensure that all tubers had been removed and graded as described in 3.2.16.

4.2.16 Statistical analysis and data handling

The data from the experiment were analysed using Genstat^{TM} 5, Release 4.1, (Lawes Agricultural Trust, IACR-Rothamsted, UK). All data was treated as described in 3.2.17.

4.2.17 Presentation of results

Since the experiment had a two by two by three factorial design, the output analysis from Genstat gave a table of means for each of the twelve treatment combinations as well as means for both cultivars, both levels of fumigant and all the granular nematicide treatments. The output analysis for the data found in Table 4.2 is shown as an example in Appendix 10. The analysis of the results showed no interaction between cultivar, fumigation and granular nematicide treatment in most cases so where this happens, the means for each of the twelve treatment combination are not shown in order to make the tables as concise as possible. However, where there was a significant interaction between cultivar, fumigation and granular nematicide treatment, the individual means for the twelve treatment combinations are shown. A complete list of results are in Appendix 12.

4.3 Results

4.3.1 Plant emergence

Plant emergence was measured at 27, 34, 38, 41 and 54 days after planting and the results for the assessments made on 27, 34, 38 days after planting are shown in Tables 4.2-4.5. An analysis of variance for repeated measurements was done to assess the effect of treatments over time and this showed that there was a significant effect of cultivar on plant emergence over time ($P < 0.001$). The emergence of Estima was significantly lower than Santé at 27, 34 and 38 days after planting but there was no difference between the emergence of both cultivars at 41 and 54 days after planting.

The time analysis showed that there was a significant effect of the application of 1,3-D on plant emergence over time ($P < 0.001$). Plant emergence was significantly increased by 1,3-D at 27, 34, 38 and 54 days after planting (Tables 4.3-4.5) but there was no difference at 41 days after planting. Oxamyl had a significant effect on plant emergence over time ($P < 0.01$). At 27 days after planting, full-rate oxamyl significantly increased plant emergence ($P = 0.011$, Table 4.3) and dose-response analysis showed that this was a linear response. Oxamyl had no effect from 34 days after planting onwards (Tables 4.4, 4.5).

At 27 days after planting, there was a significant interaction between cultivar and 1,3-D ($P = 0.007$) with emergence increased by 1,3-D more for Santé than Estima. There was also a significant interaction between cultivar and oxamyl ($P = 0.005$) with oxamyl more effective for Santé than Estima and with deviations ($P = 0.007$) that made the response non-linear.

Key findings

- Santé emerged faster than Estima but the final emergence was the same for both cultivars
- 1,3-D advanced the time of emergence
- 1,3-D advanced the time of emergence more for Santé than Estima
- at 54 days after planting, emergence was greater for plots treated with 1,3-D
- oxamyl advanced the time of emergence
- oxamyl advanced the time of emergence more for Santé than Estima
- after 54 days, final emergence was unaffected by treatment with oxamyl

Table 4.2. *The effects of cultivar, fumigation and granular nematicide treatment on the percentage of plants emerged at 27, 34, 38, 41 and 54 days after planting (DAP) and over time*

	Significance (P =)					
	27 DAP	34 DAP	38 DAP	41 DAP	54 DAP	Time
cultivar means[c]	<0.001	0.003	0.001	NS	NS	<0.001
1,3-D means[g]	<0.001	<0.001	0.034	NS	0.002	<0.001
oxamyl means[a]	0.011	NS	NS	NS	NS	<0.01
linear	0.004	NS	NS	NS	NS	-
deviations	NS	NS	NS	NS	NS	-
cultivar*1,3-D[d*b]	0.007	NS	NS	NS	NS	<0.05
cultivar*oxamyl[d*f]	0.005	NS	NS	NS	NS	<0.001
cultivar*linear	NS	NS	NS	NS	NS	-
deviations	0.007	NS	NS	NS	NS	-
1,3-D*oxamyl[e*a]	NS	NS	NS	NS	NS	NS
1,3-D*linear	NS	NS	NS	NS	NS	-
deviations	NS	NS	NS	NS	NS	-
cultivar*1,3-D*oxamyl[h]	NS	NS	NS	NS	0.046	NS
cultivar*1,3-D*linear	NS	NS	0.064	NS	0.025	-
deviations	NS	NS	NS	NS	NS	-

[a] means of either cultivars or 1,3-D treatments
[b] means for oxamyl treatments
[c] means for 1,3-D treatments and oxamyl treatments
[d] means of either 1,3-D treatments or oxamyl treatments
[e] means for cultivars
[f] means for cultivars and 1,3-D treatments
[g] means for cultivars and oxamyl treatments
[h] values not shown

Table 4.3. *The effects of cultivar, fumigation and granular nematicide treatment on the percentage of plants emerged at 27 days after planting (DAP)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	41.5	47.6	44.4	41.6	47.4	44.5
Santé	51.3	59.3	65.3	46.0	65.6	55.3
-1,3-D[e]	39.7	41.6	50.1			
+1,3-D	53.1	55.3	59.6			
oxamyl means[f]	46.4	48.4	54.8			
1,3-D means[g]				43.8	56.0	

	SED	df	CV%
cultivar means[c]	2.28		
1,3-D means[g]	2.28		
oxamyl means[a]	2.79		
cultivar*1,3-D[d*b]	3.22		
cultivar*oxamyl[d*f]	3.95		
1,3-D*oxamyl[e*a]	3.95		
cultivar*1,3-D*oxamyl[h]	5.58		
		44	17.7

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.4. *The effects of cultivar, fumigation and granular nematicide treatment on the percentage of plants emerged at 34 days after planting (DAP)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	78.9	77.4	79.4	76.7	80.5	78.6
Santé	83.9	83.4	83.0	78.9	87.9	83.4
-1,3-D[e]	77.3	77.2	78.9			
+1,3-D	85.5	83.6	83.5			
Oxamyl means[f]	81.4	80.4	81.2			
1,3-D means[g]				77.8	84.2	

	SED	df	CV%
cultivar means[c]	1.52		
1,3-D means[g]	1.52		
oxamyl means[a]	1.87		
cultivar*1,3-D[d*b]	2.15		
cultivar*oxamyl[d*f]	2.64		
1,3-D*oxamyl[e*a]	2.64		
cultivar*1,3-D*oxamyl[h]	3.73		
		44	7.3

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.5. *The effects of cultivar, fumigation and granular nematicide treatment on the percentage of plants emerged at 38 days after planting (DAP)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	81.7	81.1	80.2	79.8	82.2	81.0
Santé	85.9	84.0	85.6	83.7	86.6	85.2
-1,3-D[e]	83.0	81.3	81.0			
+1,3-D	84.6	83.8	84.8			
oxamyl means[f]	83.8	82.6	82.9			
1,3-D means[g]				81.8	84.4	

	SED	df	CV%
cultivar means[c]	1.20		
1,3-D means[g]	1.20		
oxamyl means[a]	1.47		
cultivar*1,3-D[d*b]	1.70		
cultivar*oxamyl[d*f]	2.08		
1,3-D*oxamyl[e*a]	2.08		
cultivar*1,3-D*oxamyl[h]	2.94		
		44	5.6

[a, b, c, d, e, f, g, h] see Table 4.2.

4.3.2 Percentage ground cover

The percentage of ground covered by the growing potato plants was measured at 38, 47 and 54 days after planting. An analysis of variance for repeated measurements was done to assess the effect of treatments over time and the results for the assessments are shown in Tables 4.6-4.9.

The time analysis showed that there was a significant effect of cultivar on ground cover over time ($P < 0.001$, Table 4.6). Ground cover was significantly greater for Santé than Estima at 38 days after planting ($P < 0.001$, Table 4.7) and at 47 days after planting, ($P = 0.049$, Table 4.8) but by 54 days after planting, Estima had significantly higher ground cover than Santé ($P = 0.004$, Table 4.9).

The time analysis also showed a significant effect of 1,3-D on ground cover over time ($P < 0.001$, Table 4.6). It was found that ground cover was significantly increased by 1,3-D on all assessments dates (Tables 4.6-4.9). However, the application of 1,3-D had a greater effect at 47 and 54 days after planting than at 38 days after planting indicating that the differences in ground cover were increasing with time.

Oxamyl had no effect of on ground cover over time. However, when analysed individually, it was found that ground cover was significantly linearly increased by oxamyl on all assessments dates (Tables 4.6-4.9).

There was a significant interaction between cultivar and 1,3-D over time ($P < 0.01$, Tables 4.6-4.9). Ground cover was increased more for Santé than Estima by 1,3-D on all three assessment dates. There was also a significant interaction between cultivar and oxamyl on

all three assessment dates and this had significant deviations from linearity (Tables 4.7-4.9) with oxamyl more effective for Santé than Estima. Plate 4.1 shows differences in the percentage ground covers between treatments.

Key findings

- initially, Santé had higher ground cover than Estima but this was reversed after 47 days
- 1,3-D increased the percentage ground cover on all dates when assessments were made
- 1,3-D increased ground cover more for Santé than Estima
- oxamyl increased ground cover linearly
- oxamyl increased ground cover more for Santé than Estima

Table 4.6. *The effects of cultivar, fumigation and granular nematicide treatment on Log_e percentage ground cover at 38, 47 and 54 days after planting (DAP) and over time*

	Significance (P =)			
	38 DAP	47 DAP	54 DAP	Time
cultivar means[c]	<0.001	0.049	0.004	<0.001
1,3-D means[g]	<0.001	<0.001	<0.001	<0.001
oxamyl means[a]	0.005	<0.001	<0.001	NS
linear	0.002	<0.001	<0.001	-
deviations	NS	NS	NS	-
cultivar*1,3-D[d*b]	<0.001	<0.001	0.012	<0.01
cultivar*oxamyl[d*f]	0.034	0.002	0.023	NS
cultivar*linear	NS	NS	NS	-
deviations	0.012	<0.001	0.007	-
1,3-D*oxamyl[e*a]	NS	0.060	NS	NS
1,3-D*linear	NS	0.042	NS	-
deviations	NS	NS	NS	-
cultivar*1,3-D*oxamyl[h]	NS	NS	NS	NS

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.7. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ percentage ground cover at 38 days after planting (DAP) (untransformed data in parenthesis)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	Cultivar means
Estima[d]	1.624 (5.3)	1.832 (6.4)	1.820 (6.3)	1.595 (5.1)	1.923 (6.9)	1.759 (6.0)
Santé	1.940 (7.8)	1.865 (7.5)	2.238 (10.3)	1.576 (5.3)	2.453 (11.7)	2.014 (8.5)
-1,3-D[e]	1.454 (4.4)	1.483 (4.5)	1.818 (6.7)			
+1,3-D	2.110 (8.7)	2.214 (9.4)	2.240 (9.9)			
oxamyl means[f]	1.782 (6.6)	1.848 (7.0)	2.029 (8.3)			
1,3-D means[g]				1.585 (5.2)	2.188 (9.3)	

	SED	df	CV %
38 DAP			
cultivar means[c]	0.0602		
1,3-D means[g]	0.0602		
oxamyl means[a]	0.0737		
cultivar*1,3-D[d*b]	0.0851		
cultivar*oxamyl[d*f]	0.1042		
1,3-D*oxamyl[e*a]	0.1042		
		44	12.3

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.8. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ percentage ground cover at 47 days after planting (DAP) (untransformed data in parenthesis)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	Cultivar means
Estima[d]	2.963 (20.9)	3.187 (25.8)	3.158 (24.7)	2.776 (16.6)	3.429 (31.0)	3.103 (23.8)
Santé	2.934 (22.3)	2.787 (20.2)	3.250 (29.0)	2.449 (12.9)	3.532 (34.7)	2.991 (23.8)
-1,3-D[e]	2.469 (12.1)	2.503 (13.4)	2.866 (18.8)			
+1,3-D	3.429 (31.1)	3.471 (32.6)	3.452 (34.9)			
oxamyl means[f]	2.949 (21.6)	2.987 (23.0)	3.204 (26.8)			
1,3-D means[g]				2.613 (14.8)	3.481 (32.9)	

	SED	Df	CV %
47 DAP			
cultivar means[c]	0.0553		
1,3-D means[g]	0.0553		
oxamyl means[a]	0.0677		
cultivar*1,3-D[d*b]	0.0782		
cultivar*oxamyl[d*f]	0.0958		
1,3-D*oxamyl[e*a]	0.0958		
		44	7.0

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.9. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ percentage ground cover at 54 days after planting (DAP) (untransformed data in parenthesis)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	Cultivar means
Estima[d]	3.426 (34.0)	3.628 (40.0)	3.664 (41.0)	3.222 (25.9)	3.924 (50.8)	3.572 (38.3)
Santé	3.363 (32.7)	3.315 (31.7)	3.638 (41.9)	2.971 (20.6)	3.906 (50.3)	3.439 (35.4)
-1,3-D[e]	2.930 (19.0)	3.053 (22.2)	3.308 (28.5)			
+1,3-D	3.859 (47.7)	3.891 (49.5)	3.994 (54.4)			
oxamyl means[f]	3.394 (33.3)	3.472 (35.9)	3.651 (41.4)			
1,3-D means[g]				3.097 (23.2)	3.915 (50.5)	

	SED	df	CV %
54 DAP			
cultivar means[c]	0.0445		
1,3-D means[g]	0.0445		
oxamyl means[a]	0.0546		
cultivar*1,3-D[d*b]	0.0630		
cultivar*oxamyl[d*f]	0.0772		
1,3-D*oxamyl[e*a]	0.0772		
		44	4.9

[a, b, c, d, e, f, g, h] see Table 4.2.

4.3.3 Root invasions

The invasion of potato roots by PCN at 44 days after planting was significantly lower ($P < 0.001$) for Santé than Estima when measured per g root (Tables 4.10, 4.11). 1,3-D significantly reduced invasion per g root ($P < 0.001$) but oxamyl had no effect on invasion (Tables 4.11). There was a significant interaction ($P = 0.012$) between cultivar and oxamyl with significant deviations from linearity ($P = 0.003$). There was also a significant interaction between cultivar and 1,3-D and oxamyl ($P = 0.023$) which had significant deviations from linearity ($P = 0.010$, Fig. 4.2). In fumigated plots, Estima had significantly higher invasion than Santé in the absence of oxamyl and with full-rate oxamyl. However, with half-rate oxamyl, Santé had higher invasion than Estima.

The results (Table 4.10) show that the total numbers of juveniles in Santé were less than half of the number in Estima. Since the results were skewed and no appropriate transformation could be found, no statistical analysis was done. However, the results clearly show that Santé had very few nematodes at stages J4 or J5 compared to Estima.

The invasion of potato roots by PCN at 44 days after planting was also calculated for the whole root system since growth analysis had shown that the different cultivars had significantly different root weights (Table 4.19). Invasion expressed on a whole root system was still significantly lower ($P = 0.028$) for Santé than Estima (Table 4.12). 1,3-D significantly reduced invasion per whole root ($P < 0.001$) as did oxamyl ($P = 0.024$) with the response being linear ($P = 0.010$). There was a significant interaction ($P = 0.006$) between cultivar and oxamyl with significant deviations from linearity ($P = 0.002$).

Key findings

- Santé lowered invasion per g root
- 1,3-D reduced invasion per g root
- oxamyl had no effect on invasion per g root
- Santé had a lower invasion for the whole root system
- 1,3-D reduced root invasion for the whole root system
- oxamyl reduced root invasion linearly for the whole root system

Table 4.10. *The effect of cultivar on the total root invasion (juveniles g^{-1} root) and on the numbers of juveniles at each stage in their life-cycle at 44 days after planting*

Stage in life-cycle	Estima (juveniles g^{-1} root)	Santé (juveniles g^{-1} root)
J2	2255	1650
J3	837	72
J4 female	153	2
J4 male	158	0
J5 female	8	2
J5 male	78	13
Total number	3490	1738

Table 4.11. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ root invasion (juveniles g^{-1} root) at 44 days after planting (DAP) (untransformed data in parenthesis)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	8.202 (3810)	8.349 (3435)	7.970 (3225)	8.556 (5413)	7.047 (3490)	7.802 (3490)
Santé	6.692 (1590)	6.772 (2185)	6.583 (1440)	7.791 (2660)	6.498 (817)	7.145 (1738)
1,3-D means[g]				8.174 (4037)	6.772 (1192)	

	SED	Significance (P =)	df	CV %
44 DAP				
cultivar means[c]	0.1449	<0.001		
1,3-D means[g]	0.1449	<0.001		
oxamyl means[a]	0.1775	NS		
cultivar*1,3-D[d*b]	0.2049	NS		
cultivar*oxamyl[d*f]	0.2510	0.012		
cultivar*linear		NS		
deviations		0.003		
1,3-D*oxamyl[e*a]	0.2510	NS		
cultivar*1,3-D*oxamyl	0.3549	0.023		
cultivar*1,3-D*linear		NS		
deviations		0.010		
			44	7.5

[a, b, c, d, e, f, g,] see Table 4.2

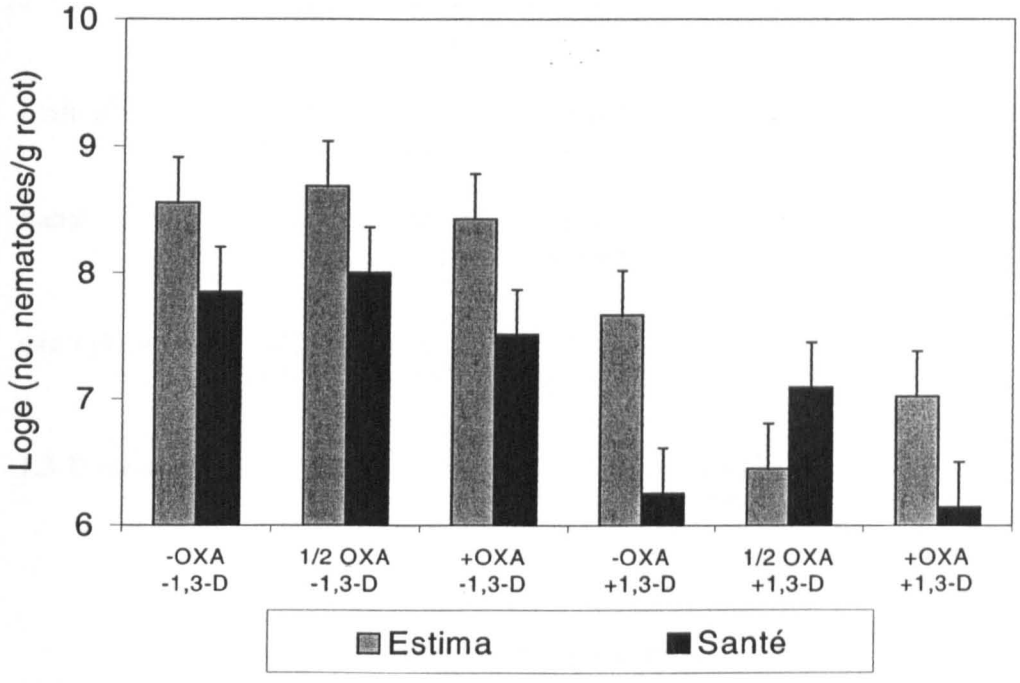

Fig. 4.2. The effect of cultivar, fumigation and granular nematicide treatment on the natural logarithm of root invasion (juveniles g^{-1} root) at 44 days after planting (DAP)

Table 4.12. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ root invasion (juvenile /whole root system) at 44 days after planting (DAP) (untransformed data in parenthesis)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	10.717 (57956)	9.951 (40094)	10.048 (36215)			10.239 (44755)
Santé	9.832 (26266)	10.357 (37373)	9.402 (18067)			9.864 (27235)
oxamyl means[f]	10.274 (42111)	10.154 (38733)	9.725 (27141)			
1,3-D means[g]				10.796 (55900)	9.307 (16090)	

	SED	Significance (P =)	df	CV %
44 DAP				
cultivar means[c]	0.1654	0.028		
1,3-D means[g]	0.1654	<0.001		
oxamyl means[a]	0.2026	0.024		
linear		0.010		
deviations		NS		
cultivar*1,3-D[d*b]	0.2339	NS		
cultivar*oxamyl[d*f]	0.2865	0.006		
cultivar*linear		NS		
deviations		0.002		
1,3-D*oxamyl[e*a]	0.2865	NS		
cultivar*1,3-D*oxamyl	0.4052	NS		
			44	6.4

[a, b, c, d, e, f, g,] see Table 4.2.

4.3.4 Growth analysis

A summary of the significant effects for each of the individual analyses done is shown in Table 4.13.

4.3.4.1 Total plant weight

Total plant weight was significantly greater ($P = 0.028$) for Santé than Estima and was significantly increased by 1,3-D ($P < 0.001$) and oxamyl ($P = 0.029$) (Tables 4.14). There was a significant interaction between cultivar and 1,3-D ($P = 0.048$) with 1,3-D increasing plant weight more for Santé than Estima. There was also a significant interaction between cultivar and oxamyl ($P = 0.004$). The total plant weight of Santé was significantly greater than Estima in the presence of full-rate oxamyl but there was no difference in weights between cultivars for the other oxamyl treatments.

4.3.4.2 Top growth

The top weight was not significantly different between cultivars but was significantly increased by 1,3-D ($P < 0.001$) and oxamyl ($P = 0.007$) (Table 4.15). Plates 4.2 and 4.3 show plants of both cultivars that were treated and untreated with 1,3-D. The response to oxamyl was linear ($P = 0.003$). There was a significant interaction between cultivar and 1,3-D ($P = 0.028$) with 1,3-D increasing weight more for Santé than Estima. There was a significant interaction between cultivar and oxamyl ($P = 0.003$) with the response showing deviations from linearity ($P = 0.002$).

4.3.4.3 Stolon weight

The weight of stolons was significantly greater for Santé than Estima ($P < 0.001$) (Table 4.16). 1,3-D significantly increased stolon weight ($P < 0.001$) but oxamyl had no effect.

Table 4.13. *The effects of cultivar, fumigation and granular nematicide treatment on plant growth at 44 days after planting (DAP)*

	total plant wt. (g)	top wt. (g)	stolon wt. (g)	no. of stems	stem wt. (g)	root wt. (g)	shoot /root wt. ratio)
cultivar means[c]	0.028	NS	<0.001	NS	0.017	<0.001	NS
1,3-D means[g]	<0.001	<0.001	<0.001	0.014	0.001	NS	<0.001
oxamyl·means[a]	0.029	0.007	NS	NS	NS	0.005	<0.001
linear	0.014	0.003	NS	NS	NS	0.001	<0.001
deviations	NS	0.251	NS	NS	NS	NS	NS
cultivar*1,3-D[d*b]	0.048	0.028	NS	NS	NS	NS	NS
cultivar*oxamyl[d*f]	0.004	0.003	NS	<0.001	0.005	NS	0.004
cultivar*linear	NS	NS	NS	<0.001	0.002	NS	NS
deviations	0.003	0.002	NS	NS	NS	NS	0.001
1,3-D*oxamyl[e*a]	NS	NS	NS	NS	NS	NS	NS
cultivar*1,3-D*oxamyl[h]	NS	NS	NS	NS	NS	NS	NS

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.14. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ total plant weight (g) at 44 days after planting (DAP) (untransformed data in parenthesis)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	4.879	5.064	4.951	4.709	5.221	4.965
	(139.3)	(167.9)	(150.1)	(115.0)	(189.6)	(152.4)
Santé	5.042	4.917	5.397	4.724	5.513	5.119
	(173.2)	(153.6)	(241.4)	(123.2)	(255.6)	(189.4)
oxamyl means[f]	4.960	4.991	5.174			
	(156.3)	(160.8)	(195.8)			
1,3-D means[g]				4.716	5.367	
				(119.1)	(222.8)	

	SED	df	CV %
cultivar means[c]	0.0680		
1,3-D means[g]	0.0680		
oxamyl means[a]	0.0833		
cultivar*1,3-D[d*b]	0.0962		
cultivar*oxamyl[d*f]	0.1178		
		44	5.2

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.15. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ top weight (g) at 44 days after planting (DAP) (untransformed data in parenthesis)*

	oxamyl[a]			1,3-D[b]	
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D
Estima[d]	4.549	4.831	4.720	4.375	5.025
	(105.1)	(137.3)	(122.8)	(85.3)	(158.1)
Santé	4.674	4.507	5.159	4.259	5.301
	(130.3)	(111.2)	(196.9)	(83.3)	(209.0)
oxamyl means[f]	4.611	4.669	4.940		
	(117.7)	(124.3)	(159.8)		
1,3-D means[g]				4.317	5.163
				(84.3)	(183.6)

	SED	df	CV %
1,3-D means[g]	0.0862		
oxamyl means[a]	0.1056		
cultivar*1,3-D[d*b]	0.1219		
cultivar*oxamyl[d*f]	0.1493		
		44	7.0

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.16. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ stolon weight (g) at 44 days after planting (DAP) (untransformed data in parenthesis)*

	1,3-D[b]		cultivar[c]
	-1,3-D	+1,3-D	cultivar means
Estima[d]			0.808 (2.51)
Santé			1.683 (6.09)
1,3-D means[g]	0.967 (3.16)	1.524 (5.43)	

	SED	df	CV %
cultivar means[c]	0.0917		
1,3-D means[g]	0.0917		
		44	28.5

[a, b, c, d, e, f, g, h] see Table 4.2.

untreated treated with 1,3-D

Plate 4.2. Plants of Santé at 60 days after planting.

untreated treated with 1,3-D

Plate 4.3. Plants of Estima at 60 days after planting.

4.3.4.4 Number and weight of stems

The number of stems was not significantly different between cultivars or with the use of oxamyl but was significantly increased by 1,3-D ($P = 0.014$) (Table 4.17). There was a significant interaction ($P < 0.001$) between cultivar and oxamyl with the number of stems decreased for Estima by oxamyl and increased for Santé. The response was linear ($P < 0.001$).

The weight of stems was significantly less for Estima than Santé ($P = 0.017$) (Table 4.18). 1,3-D significantly increased the weight of stems ($P = 0.001$). There was a significant interaction ($P = 0.005$) between cultivar and oxamyl with the weight of stems decreased by oxamyl for Estima and increased for Santé. This response was linear ($P = 0.002$).

4.3.4.5 Root weight

Root weight was significantly less for Estima than Santé ($P < 0.001$) (Table 4.19). The root weights of plants untreated by oxamyl were significantly higher than those treated ($P = 0.005$). This response was linear ($P = 0.001$).

4.3.4.6 Shoot weight /root weight

The ratio of shoot weight /root weight was significantly decreased by the use of 1,3-D ($P < 0.001$) and by the use of oxamyl in a linear way ($P < 0.001$) (Table 4.20). There was an interaction between cultivar and oxamyl ($P = 0.004$) which showed deviations from linearity ($P = 0.001$). The use of half-rate oxamyl significantly increased the shoot weight /root weight ratio for Estima but not for Santé. The other levels of oxamyl gave no significant response.

4.3.4.7 Relationship between top weight and ground cover

A correlation was done between top fresh weight (at 44 days after planting) and percentage ground cover (at 47 days after planting) for Estima and Santé. The results (Fig. 4.3) show that Estima had a r^2 of 0.454 and Santé had a stronger relationship with a r^2 of 0.863. The results show as increase in ground cover with an increase in top weight as would be expected.

Key findings

- total plant weight was greater for Santé than Estima
- top weight was not significantly different between cultivars
- 1,3-D increased total plant weight and top weight
- oxamyl increased total plant weight and top weight
- 1,3-D increased stolon weight, number of stems and weight of stems
- Estima had lower weights of stems and root weights than Santé
- root weight was lower for plants treated with oxamyl
- correlation between increase in ground cover and increase in top weight

Table 4.17. The effects of cultivar, fumigation and granular nematicide treatment on the number of stems at 44 days after planting (DAP)

	oxamyl[a]			1,3-D[b]	
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D
Estima[d]	4.65	4.40	4.05		
Santé	3.80	4.05	5.45		
1,3-D means[g]				4.10	4.70

	SED	df	CV %
1,3-D means[g]	0.234		
cultivar*oxamyl[d*f]	0.405		
		44	20.6

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.18. *The effects of cultivar, fumigation and granular nematicide treatment on the weight of stems (g) at 44 days after planting (DAP)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	18.03	16.80	15.91			16.38
Santé	17.05	18.03	21.06			18.71
1,3-D means[g]				15.94	19.16	

	SED	df	CV %
cultivar means[c]	0.942		
1,3-D means[g]	0.942		
cultivar*oxamyl[d*f]	1.332		
		44	20.8

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.19. *The effects of cultivar, fumigation and granular nematicide treatment on root weight (g) at 44 days after planting (DAP)*

	oxamyl[a]			cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	cultivar means
Estima[d]				11.79
Santé				15.62
oxamyl means[f]	15.26	13.96	11.89	

	SED	df	CV %
cultivar means[c]	0.794		
oxamyl means[a]	0.972		
		44	22.4

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.20. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ (shoot weight /root weight) at 44 days after planting (DAP) (untransformed data in parenthesis)*

	oxamyl[a]			1,3-D[b]	
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D
Estima[d]	1.943 (8.65)	2.450 (13.62)	2.396 (12.86)		
Santé	1.895 (8.5)	1.700 (7.37)	2.587 (14.49)		
oxamyl means[f]	1.919 (8.57)	2.075 (10.49)	2.492 (13.68)		
1,3-D means[g]				1.695 (6.65)	2.628 (15.18)

	SED	df	CV %
1,3-D means[g]	0.1117		
oxamyl means[a]	0.1367		
cultivar*oxamyl[d*f]	0.1934		
		44	20.0

[a, b, c, d, e, f, g, h] see Table 4.2.

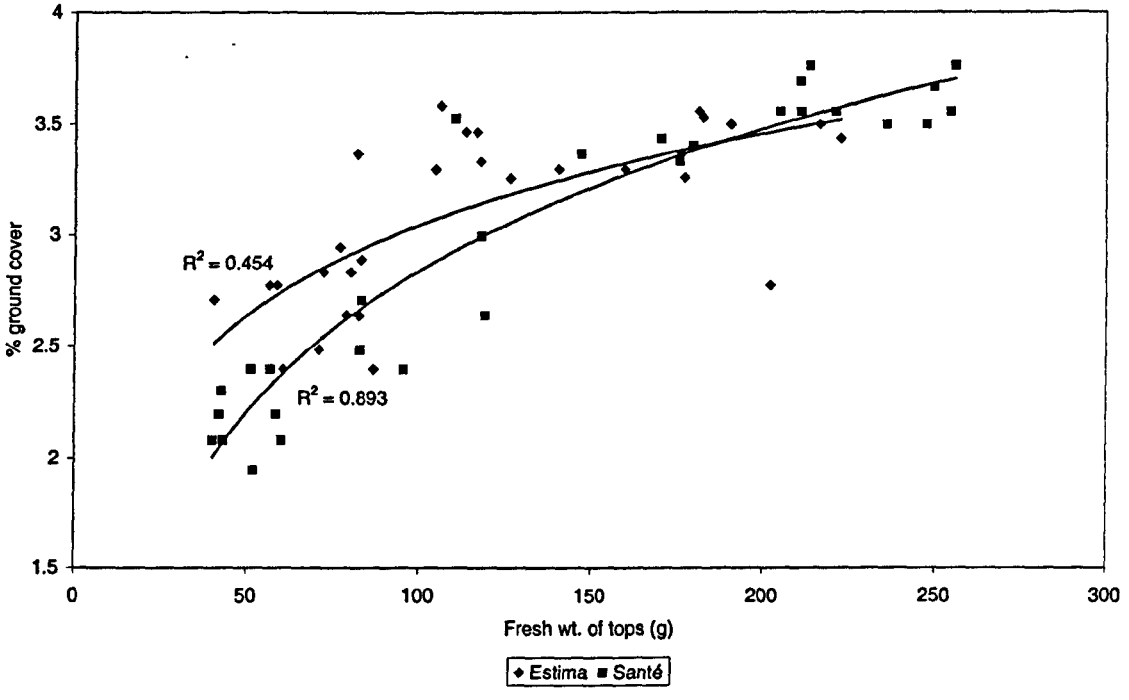

Fig. 4.3. The relationship between top fresh weight (at 44 days after planting) and percentage ground cover (at 47 days after planting) for Estima and Santé

4.3.5 Incidence of *R. solani*

The results for the assessment of the incidence of *R. solani* (Table 4.21) showed that as in the first experiment, the plots that were treated with 1,3-D had a lower incidence of infection although again this was not statistically significant. There was no significant reduction in levels of *R. solani* between varieties or with the use of oxamyl.

Table 4.21. *The effects of fumigation with 1,3-D on the incidence of stems diseased by* Rhizoctonia solani *(per plant) on the potato cultivars Estima and Santé at 44 days after planting (DAP)*

	oxamyl[a]			1,3-D[b]		variety[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	variety means
Estima[d]	0.173	0.228	0.061	0.170	0.139	0.154
Santé	0.094	0.139	0.129	0.134	0.108	0.121
-1,3-D[e]	0.147	0.208	0.100			
+1,3-D	0.121	0.159	0.090			
oxamyl means[f]	0.134	0.184	0.095			
1,3-D means[g]				0.152	0.123	

	SED	Significance (P =)	df	CV %
44 DAP				
cultivar means[c]	0.0513	NS		
1,3-D means[g]	0.0513	NS		
oxamyl means[a]	0.0628	NS		
cultivar*1,3-D[d*b]	0.0725	NS		
cultivar*oxamyl[d*f]	0.0888	NS		
1,3-D*oxamyl[e*a]	0.0888	NS		
cultivar*1,3-D*oxamyl[h]	0.1256	NS		
			44	17.1

[a, b, c, d, e, f, g] see Table 4.2.

4.3.6 Yield of tubers at harvest

A summary of the significant effects for each of the individual analyses done is shown in Table 4.22.

4.3.6.1 Yield of tubers less than 45 mm

The weight of tubers less than 45 mm was significantly less for Santé than Estima ($P < 0.001$) (Table 4.23). 1,3-D significantly increased the yield of tubers ($P < 0.001$) as did oxamyl ($P = 0.022$). The response to oxamyl was linear ($P = 0.006$). There was a significant interaction between cultivar and oxamyl ($P = 0.043$) with oxamyl increasing the yield for Santé but not Estima. There was a significant interaction between 1,3-D and oxamyl ($P = 0.031$) with oxamyl increasing the yield only when 1,3-D was not applied. There was a significant interaction ($P = 0.030$) between cultivar and 1,3-D and oxamyl. Oxamyl had no significant effect on yield when used 1,3-D on Estima. However, oxamyl significantly increased yields when used with 1,3-D for Santé and when used without 1,3-D for both cultivars.

4.3.6.2 Yield of tubers of 45-65 mm

The yield of tubers of 45-65 mm was significantly less for Estima than Santé ($P < 0.001$) (Table 4.24). 1,3-D significantly increased the yield of tubers ($P < 0.001$) as did oxamyl ($P < 0.001$). The response to oxamyl was linear ($P < 0.001$). There was a significant interaction ($P < 0.001$) between cultivar and 1,3-D with a greater response from Estima to the use of 1,3-D.

4.3.6.3 Yield of tubers of 65-85 mm

The yield of tubers of 65-85 mm was significantly less for Estima than Santé ($P < 0.001$) (Table 4.25). 1,3-D significantly increased ($P < 0.001$) the yield of tubers but oxamyl had no effect.

Table 4.22. The effects of cultivar, fumigation and granular nematicide treatment on yield of tubers of individual grades, ware, total and percentage ware grades

	< 45 mm	45-65 mm	65-85 mm	ware grade	Total yield	% ware yield
cultivar means[c]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1,3-D means[g]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
oxamyl means[a]	0.022	<0.001	NS	0.018	0.005	NS
linear	0.006	<0.001	NS	0.005	0.001	NS
deviations	NS	NS	NS	NS	NS	NS
cultivar*1,3-D[d*b]	NS	<0.001	NS	0.037	0.035	<0.001
cultivar*oxamyl[d*f]	0.043	NS	NS	NS	NS	0.013
cultivar*linear	NS	NS	NS	NS	NS	0.004
deviations	0.035	NS	NS	NS	NS	NS
1,3-D*oxamyl[e*a]	0.031	NS	NS	NS	NS	NS
1,3-D*linear	0.011	NS	NS	NS	NS	NS
deviations	NS	NS	NS	NS	NS	NS
cultivar*1,3-D*oxamyl	0.030	NS	NS	NS	NS	NS
cultivar*1,3-D*linear	0.009	NS	NS	NS	NS	NS
deviations	NS	NS	NS	NS	NS	NS

[a, b, c, d, e, f, g] see Table 4.2.

Table 4.23. *The effects of cultivar, fumigation and granular nematicide treatment on yield of tubers (t ha^{-1}) less than 45 mm*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	6.4	7.2	6.9			6.8
Santé	2.7	2.7	4.0			3.1
-1,3-D[e]	3.4	4.0	5.1			
+1,3-D	5.7	5.9	5.8			
oxamyl means[f]	4.5	4.9	5.4			
1,3-D means[g]				4.2	5.8	

	-1,3-D untreated	-1,3-D ½ oxamyl	-1,3-D oxamyl	+1,3-D untreated	+1,3-D ½ oxamyl	+1,3-D Oxamyl
Estima	4.7	6.2	6.9	8.1	8.2	6.9
Santé	2.0	1.8	3.4	3.3	3.5	4.7

	SED	df	CV %
cultivar means[c]	0.26		
1,3-D means[g]	0.45		
oxamyl means[a]	0.32		
cultivar*oxamyl[d*f]	0.45		
1,3-D*oxamyl[e*a]	0.45		
cultivar*1,3-D*oxamyl	0.64		
		44	20.5

[a, b, c, d, e, f, g] see Table 4.2.

Table 4.24. *The effects of cultivar, fumigation and granular nematicide treatment on yield of tubers (t ha^{-1}) of 45-65 mm*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]				7.0	26.3	16.7
Santé				18.3	30.8	24.5
oxamyl means[f]	18.7	20.0	23.1			
1,3-D means[g]				12.6	28.6	

	SED	df	CV%
cultivar means[c]	0.85		
1,3-D means[g]	0.85		
oxamyl means[a]	1.03		
cultivar*1,3-D[d*b]	1.20		
		44	15.9

[a, b, c, d, e, f, g] see Table 4.2.

Table 4.25. *The effects of cultivar, fumigation and granular nematicide treatment on yield of tubers (t ha^{-1}) of 65-85 mm*

	1,3-D[b]		cultivar[c]
	-1,3-D	+1,3-D	cultivar means
Estima[d]			2.1
Santé			16.7
1,3-D means[g]	6.8	12.1	

	SED	df	CV%
cultivar means[c]	0.84		
1,3-D means[g]	0.84		
		44	34.5

[a, b, c, d, e, f, g] see Table 4.2.

4.3.6.4 Yield of tubers of ware grade

The yield of ware grade tubers (45-85 mm) was significantly less for Estima than Santé ($P < 0.001$) (Table 4.26). 1,3-D significantly increased ($P < 0.001$) the ware yield as did oxamyl ($P = 0.018$). There was a significant interaction between cultivar and 1,3-D ($P = 0.037$) with 1,3-D having a greater effect on Estima.

4.3.6.5 Total yield

The total yield of tubers was significantly less for Estima than Santé ($P < 0.001$) (Table 4.27). 1,3-D significantly increased the total yield ($P < 0.001$) as did oxamyl ($P = 0.005$). The response to oxamyl was linear ($P < 0.001$) and full-rate oxamyl increased yields significantly more than half-rate oxamyl. There was a significant interaction ($P = 0.035$) between cultivar and 1,3-D and oxamyl with 1,3-D having a greater effect on Estima. The effects of cultivar, fumigation and granular nematicide treatment on the total yield and yield of individual grades for each treatment is shown in Fig. 4.4. For Estima, yields of all grades of tuber are increased by 1,3-D with the greatest increase in the 45-65 mm grade. For Santé, the yields of 45-65 mm and 65-85 mm are increased by fumigation. Estima has greater yield less than 45 mm. The yield of Santé in the absence of 1,3-D is similar to that of Estima with fumigation. However, there is a greater proportion of yield that is non-ware grade for Estima.

4.3.6.6 Percentage of tubers of ware grade

The percentage of tubers which were of ware grade (45-85 mm) was significantly less for Estima than Santé ($P < 0.001$) (Table 4.28). 1,3-D significantly increased the percentage of tubers of ware grade ($P < 0.001$) but oxamyl had no effect. There was a significant interaction ($P < 0.001$) between cultivar and 1,3-D; the percentage ware grade from using

1,3-D was increased with Estima but was unchanged with Santé. There was a significant interaction between cultivar and oxamyl (P = 0.013); half and full-rate oxamyl both increased yields with Estima but not with Santé. The response by Estima was linear (P = 0.004).

4.3.6.7 Mean tuber weight

The mean tuber weight of Santé was greater than that of Estima ($P < 0.001$) (Table 4.29). 1,3-D significantly increased the mean weight of tubers ($P < 0.001$) but oxamyl had no effect. There was a significant interaction between 1,3-D and cultivar. 1,3-D significantly increased the tuber weight for Estima but not Santé. There was also a significant interaction between oxamyl and cultivar. Estima had a significantly higher mean tuber weight with the use of half and full-rate oxamyl (P = 0.012), but the mean tuber weight of Santé was unchanged by half-rate oxamyl and was significantly less with full-rate oxamyl.

Key findings

- Santé had a lower yield of tubers of less than 45mm
- Estima had lower yields of tubers of 45-65 mm and 65-85 mm
- Estima had lower ware and total yields
- 1,3-D and oxamyl increased the yield of tubers less than 45 mm and of 45-65 mm
- 1,3-D also increased yield of tubers of 65-85 mm
- 1,3-D and oxamyl increased ware and total yields
- Santé had a higher percentage of tubers of ware grade
- 1,3-D increased the percentage of tubers of ware grade but oxamyl had no effect

Table 4.26. The effects of cultivar, fumigation and granular nematicide treatment on yield of tubers (t ha^{-1}) of ware grade (45-85 mm)

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]				7.2	30.4	18.8
Santé				31.8	50.8	41.3
oxamyl means[f]	28.5	29.6	32.0			
1,3-D means[g]				19.5	40.6	

	SED	df	CV%
cultivar means[c]	0.99		
1,3-D means[g]	0.99		
oxamyl means[a]	1.21		
		44	12.8

[a, b, c, d, e, f, g] see Table 4.2.

Table 4.27. The effects of cultivar, fumigation and granular nematicide treatment on total yield (t ha^{-1}) of tubers

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]				13.1	38.2	25.6
Santé				34.2	54.6	44.4
oxamyl means[f]	33.0	34.6	37.5			
1,3-D means[g]				23.6	46.4	

	SED	df	CV%
cultivar means[c]	1.07		
1,3-D means[g]	1.07		
oxamyl means[a]	1.31		
		44	11.8

[a, b, c, d, e, f, g] see Table 4.2.

Table 4.28. *The effects of cultivar, fumigation and granular nematicide treatment on the percentage of tubers of ware grade*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	60.9	66.9	69.5	52.0	79.5	64.8
Santé	93.9	93.9	91.4	93.1	93.0	93.0
1,3-D means[g]				72.6	86.3	

	SED	df	CV%
cultivar means[c]	1.46		
1,3-D means[g]	1.46		
cultivar*1,3-D[d*b]	2.06		
cultivar*oxamyl[d*f]	2.63		
		44	7.1

[a, b, c, d, e, f, g] see Table 4.2.

Table 4.29. *The effects of cultivar, fumigation and granular nematicide treatment on the mean tuber weight*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	0.0521	0.0602	0.0617	0.0393	0.0767	0.0580
Santé	0.1147	0.1165	0.1010	0.1118	0.1097	0.1108
1,3-D means[g]				0.0756	0.0932	

	SED	Significance (P =)	df	CV %
≤ 45 mm				
cultivar means[c]	0.00316	<0.001		
1,3-D means[g]	0.00316	<0.001		
cultivar*1,3-D[d*b]	0.00446	<0.001		
cultivar*oxamyl[d*f]	0.00547	0.012		
cultivar*linear		0.004		
deviations		NS		
			44	14.5

[a, b, c, d, e, f, g] see Table 4.2.

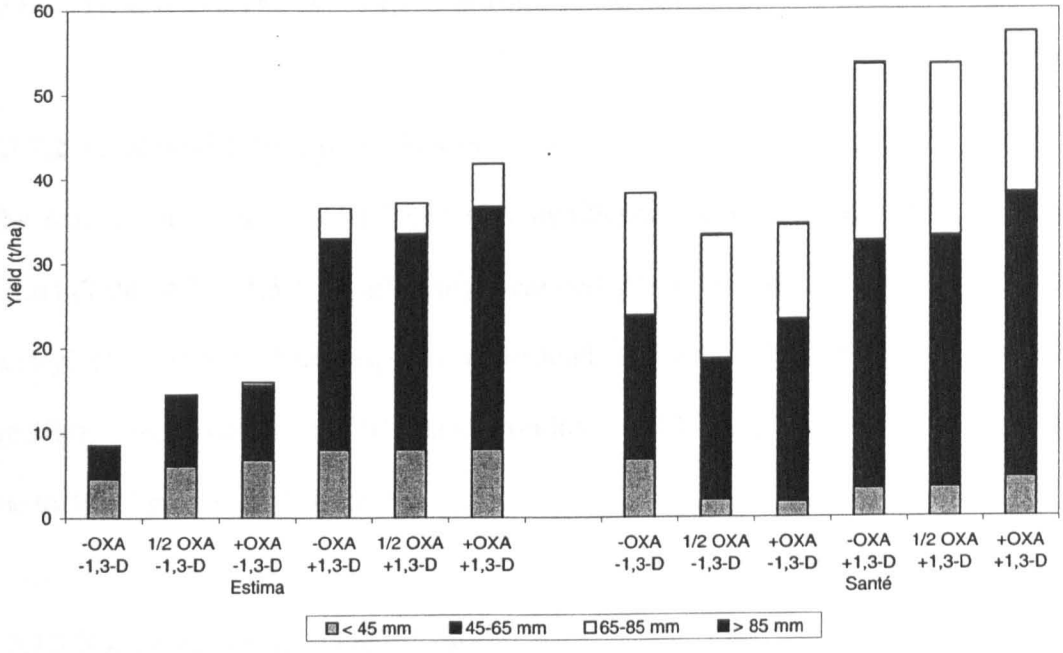

Fig. 4.4. The effects of cultivar, fumigation and granular nematicide treatment on the total yield and yield of individual grades (t ha^{-1})

4.3.7 Number of tubers at harvest

A summary of the significant effects for each of the individual analyses done is shown in Table 4.30.

4.3.7.1 Number of tubers less than 45 mm

The number of tubers less than 45 mm was significantly greater for Estima than Santé ($P < 0.001$) (Table 4.31). The use of 1,3-D or oxamyl had no effect.

4.3.7.2 Number of tubers of 45-65 mm

The number of tubers of 45-65 mm was significantly less for Estima than Santé ($P < 0.001$) (Table 4.32). 1,3-D significantly increased ($P < 0.001$) the number of tubers as did oxamyl ($P = 0.002$). The response to oxamyl was linear ($P < 0.001$). There was a significant interaction ($P < 0.001$) between cultivar and 1,3-D ($P = 0.050$) with the increase due to 1,3-D greater for Estima.

4.3.7.3 Number of tubers of 65-85 mm

The number of tubers of 65-85 mm was significantly less for Estima than Santé ($P < 0.001$) (Table 4.33). 1,3-D significantly increased the number of tubers ($P < 0.001$) but oxamyl had no effect. There was a significant interaction between cultivar and 1,3-D ($P = 0.042$) with the increase in tuber number due to 1,3-D greater for Estima.

4.3.7.4 Number of tubers of ware grade

The number of tubers of ware grade (45-85 mm) was significantly less for Estima than Santé ($P < 0.001$) (Table 4.34). 1,3-D significantly increased the number of tubers ($P < 0.001$) as did full-rate oxamyl ($P = 0.002$). The response to oxamyl was linear ($P < 0.001$).

The greatest effect on the number of ware grade tubers was by application of 1,3-D which increased the number for both Estima and Santé (Fig. 4.5).

4.3.7.5 Total number of tubers

The total number of tubers was similar for both cultivars (Table 4.35). 1,3-D significantly increased the number of tubers ($P < 0.001$) as did full-rate oxamyl ($P = 0.003$). The response to oxamyl was linear ($P = 0.001$). The effects of each treatment is shown in Fig 4.5. The greatest effect was the application of 1,3-D which was seen to increase the total numbers of tubers for both cultivars.

4.3.7.6 Percentage of tubers of ware grade

The percentage number of tubers which were of ware grade was significantly less for Estima than Santé ($P < 0.001$) (Table 4.36). 1,3-D significantly increased this percentage ($P < 0.001$). There was a significant interaction between cultivar and 1,3-D ($P < 0.001$); the increase due to 1,3-D was significant for Estima but not Santé.

Key findings

- Estima had a greater number of tubers less than 45 mm than Santé
- 1,3-D or oxamyl had no effect on the number of tubers less than 45 mm
- 1,3-D increased the numbers of tubers of 45-65 mm and 65-85 mm
- oxamyl increased the numbers of tubers of 45-65 mm
- Santé had more ware grade tubers and a higher percentage of ware grade than Estima
- 1,3-D and oxamyl both increased the numbers of tubers of ware grade and the total numbers of tubers
- 1,3-D increased the percentage of tubers of ware grade

Table 4.30. *The effects of cultivar, fumigation and granular nematicide treatment on the number of tubers*

	< 45 mm	45-65 mm	65-85 mm	ware grade	total yield	% ware grade
cultivar means[c]	<0.001	<0.001	<0.001	<0.001	0.721	<0.001
1,3-D means[g]	NS	<0.001	<0.001	<0.001	<0.001	<0.001
oxamyl means[a]	NS	0.002	NS	0.002	0.003	NS
linear	NS	<0.001	NS	<0.001	0.001	NS
deviations	NS	NS	NS	NS	NS	NS
cultivar*1,3-D[d*b]	NS	0.050	0.042	0.182	NS	<0.001
cultivar*oxamyl[d*f]	NS	NS	NS	NS	NS	NS
1,3-D*oxamyl[e*a]	NS	NS	NS	NS	NS	NS
cultivar*1,3-D*oxamyl[h]	NS	NS	NS	NS	NS	NS

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.31. The effects of cultivar, fumigation and granular nematicide treatment on the number of tubers less than 45 mm (000's ha^{-1})

	cultivar means
Estima[d]	234.8
Santé	100.8

SED	df	CV %
10.85	44	25.0

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.32. *The effects of cultivar, fumigation and granular nematicide treatment on the number of tubers of 45-65 mm (000's ha^{-1})*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]				87.2	254.4	170.8
Santé				170.1	294.8	232.5
-1,3-D[e]	104.4	126.3	155.3			
+1,3-D	258.9	261.5	303.4			
oxamyl means[f]	181.6	193.9	229.3			
1,3-D means[g]				128.7	274.6	

	SED	df	CV %
cultivar means[c]	10.56		
1,3-D means[g]	10.56		
oxamyl means[a]	12.94		
cultivar*1,3-D[d*b]	14.94		
		44	20.3

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.33. *The effects of cultivar, fumigation and granular nematicide treatment on the number of tubers of 65-85 mm (000's ha^{-1})*

	1,3-D[b]		cultivar[c]
	-1,3-D	+1,3-D	cultivar means
Estima[d]	0.9	19.7	10.3
Santé	60	92.8	76.4
1,3-D means[g]	30.5	56.2	

	SED	df	CV %
cultivar means[c]	3.33		
1,3-D means[g]	3.33		
cultivar*1,3-D[d*b]	4.71		
		44	29.8

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.34. *The effects of cultivar, fumigation and granular nematicide treatment on the number of tubers of ware grade (45-85 mm) (000's ha^{-1})*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]						181.1
Santé						309.0
oxamyl means[f]	226.0	236.9	272.2			
1,3-D means[g]				159.2	330.9	

	SED	df	CV %
cultivar means[c]	10.56		
1,3-D means[g]	10.56		
oxamyl means[a]	12.93		
		44	16.7

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.35. *The effects of cultivar, fumigation and granular nematicide treatment on the total number of tubers (000's ha^{-1})*

	oxamyl[a]			1,3-D[b]	
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D
oxamyl means[f]	384.1	399.4	455.1		
1,3-D means[g]				322.5	503.2

	SED	df	CV %
1,3-D means[g]	16.92		
oxamyl means[a]	20.73		
		44	15.9

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.36. *The effects of cultivar, fumigation and granular nematicide treatment on the percentage of the number of tubers that were of ware grade (45-85 mm)*

	1,3-D[b]		cultivar[c]
	-1,3-D	+1,3-D	cultivar means
Estima[d]	26.4	54.8	40.6
Santé	73.8	77.0	75.4
1,3-D means[g]	50.12	65.91	

	SED	df	CV %
cultivar means[c]	1.69		
1,3-D means[g]	1.69		
cultivar*1,3-D[d*b]	2.39		
		44	11.3

[a, b, c, d, e, f, g, h] see Table 4.2.

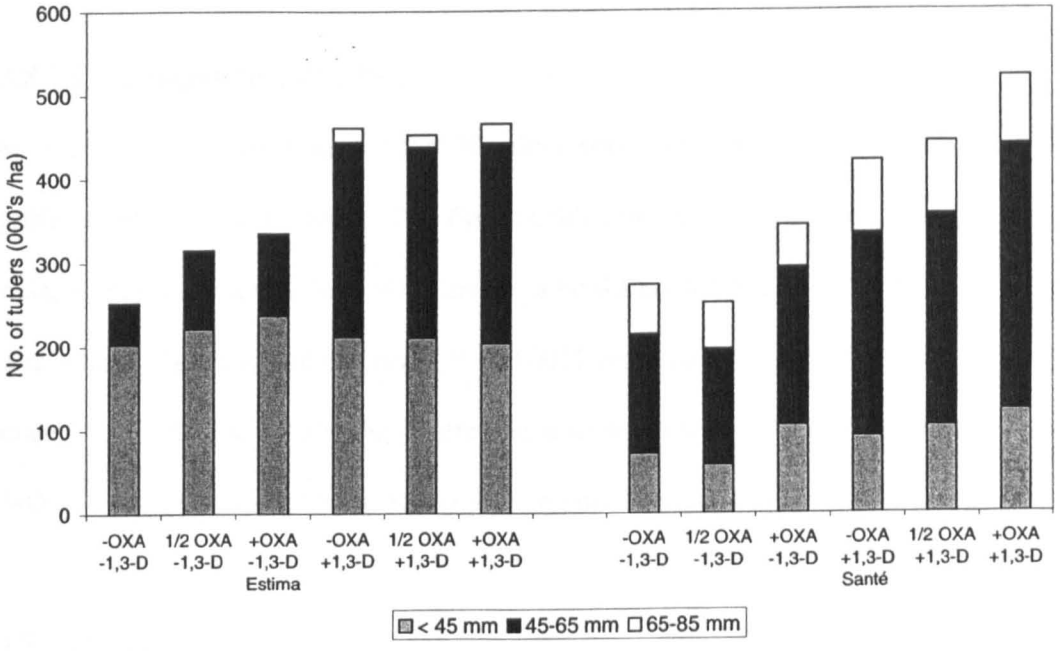

Fig. 4.5. The effects of cultivar, fumigation and granular nematicide treatment on the total number of tubers and the numbers of tubers in individual grades (000's ha^{-1})

4.3.8 Nematode population densities

4.3.8.1 Initial population densities

The initial population densities (Pi) for the experiment were not significantly different between treatments (Table 4.37). The overall mean for the experiment was 190 eggs g^{-1} soil. The CV of 13.5 % would be expected from a field experiment and demonstrates that the variability was not great.

4.3.8.2 Final population densities

The use of Pi as a covariate for the Pf values was tried but it was found that it had no significant effect as a covariate. The final population density (Pf) was significantly lower for Santé than Estima (Table 4.38). There was no difference due to either 1,3-D or oxamyl. There was a significant interaction ($P < 0.001$) between cultivar and 1,3-D; the Pf was reduced for Santé but not Estima. There was a significant interaction ($P = 0.016$) between 1,3-D and oxamyl; oxamyl reduced the Pf when used with 1,3-D but not without it.

4.3.8.3 Pf/Pi ratios

It was found that Pi had a significant effect as a covariate for the Pf/Pi ratios ($P < 0.001$). The Pf/Pi ratios were multiplied by a factor of 101 before transformation in order that the final transformed values would be positive (Little & Hills, 1978). The multiplication ratio (Pf/Pi) was significantly lower for Santé than Estima (Table 4.39). 1,3-D or oxamyl had no significant effect on the Pf/Pi ratios. There was a significant interaction between cultivar and 1,3-D ($P < 0.001$); Pf was significantly reduced for Santé but not Estima. There was also a significant interaction between 1,3-D and oxamyl ($P = 0.019$) and the analysis showed that this was a linear response ($P = 0.006$). In the presence of full-rate oxamyl, plots that were treated with 1,3-D had significantly lower PF/Pi ratios than untreated plots.

It was also found that in the presence of 1,3-D, plots that were treated with full-rate oxamyl had significantly lower PF/Pi ratios than plots that were not treated with oxamyl.

Key findings

- Santé had a significantly lower Pf/Pi ratios than Estima
- 1,3-D or oxamyl had no effect on the Pf/Pi ratios
- there was an interaction between 1,3-D and oxamyl with reduced multiplication by the use of both 1,3-D and full-rate oxamyl

Table 4.37. *The initial population densities (Pi) (eggs g^{-1} soil) of plots before treatment with fumigant or granular nematicides*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	Half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]	158	193	203	190	181	185
Santé	231	198	154	180	208	194
-1,3-D[e]	158	193	203			
+1,3-D	231	198	154			
oxamyl means[f]	195	196	178			
1,3-D means[g]				185	194	
Overall mean for all plots		190				

	SED	Significance (P =)	df	CV %
Pi				
cultivar means[c]	29.2	NS		
1,3-D means[g]	29.2	NS		
oxamyl means[a]	35.7	NS		
cultivar*1,3-D[d*b]	41.3	NS		
cultivar*oxamyl[d*f]	50.5	NS		
1,3-D*oxamyl[e*a]	50.5	NS		
cultivar*1,3-D*oxamyl[h]	71.5	NS		
			44	59.5

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.38. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ Pf (untransformed data in parenthesis)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]				5.007 (173)	5.322 (241)	5.165 (207)
Santé				2.515 (16)	1.803 (7)	2.159 (11)
-1,3-D[e]	3.608 (99)	3.795 (99)	3.881 (85)			
+1,3-D	4.014 (181)	3.456 (90)	3.218 (101)			

	SED	Significance (P =)	df	CV %
Log$_e$ Pf				
cultivar means[c]	0.1480	<0.001		
cultivar*1,3-D[d*b]	0.2093	0.001		
1,3-D*oxamyl[e*a]	0.2563	0.016		
1,3-D*linear		0.005		
deviations		0.506		
			44	15.7

[a, b, c, d, e, f, g, h] see Table 4.2.

Table 4.39. *The effects of cultivar, fumigation and granular nematicide treatment on Log$_e$ Pf/Pi (adjusted for Pi as a covariate) (untransformed data in parentheses)*

	oxamyl[a]			1,3-D[b]		cultivar[c]
	no oxamyl	half-rate oxamyl	full-rate oxamyl	-1,3-D	+1,3-D	cultivar means
Estima[d]				4.57	4.92	4.74
				(1.39)	(1.97)	(1.68)
Santé				2.08	1.23	1.67
				(0.12)	(0.04)	(0.08)
-1,3-D[e]	3.147	3.363	3.460			
	(1.22)	(0.56)	(0.49)			
+1,3-D	3.537	2.996	2.728			
	(1.11)	(1.06)	(0.84)			

	SED	Significance (P =)	df	CV %
Log$_e$ (Pf/Pi)				
cultivar means[c]	0.1578	<0.001		
cultivar*1,3-D[d*b]	0.2234	<0.001		
1,3-D*oxamyl[e*a]	0.2751	0.019		
linear		0.006		
deviations		NS		
covariate (Pi)	0.000814[j]	<0.001		
			43	19.0

[a, b, c, d, e, f, g, h] see Table 4.2.

[j] SE

4.4 Discussion

The results of the experiment show that an integrated approach to nematode control on heavily infested sites can lead to significant decreases in nematode population levels and give economic yield benefits.

4.4.1 Plant emergence

The faster emergence of Santé compared to Estima was unexpected since it is a maincrop cultivar and Estima is a second early cultivar. The results for the root invasion (Table 4.10) show a higher degree of invasion of Estima. The damage caused by invasion could explain its late emergence and lower ground cover compared to Santé. There may be other factors involved, such as the physiological age of the seed and the way that it was treated. However, the final emergence was the same for both cultivars.

1,3-D advanced emergence for both cultivars. 1,3-D but more for Santé than Estima. In addition, the final emergence was higher for plots treated with 1,3-D. The experimental site had an initial population density of 190 eggs g^{-1} soil and this level of infestation may have caused a small number of plants to fail to emerge in plots untreated by 1,3-D.

The rate of emergence was advanced by oxamyl but was advanced more for Santé than Estima, as with 1,3-D. By 34 days after planting, the emergence was unaffected by oxamyl treatment. Grove *et al.* (1999*b*) also found a benefit from the rate of plant emergence from oxamyl. The effect of oxamyl was shorter-lived than the effect of 1,3-D. The greater effect of 1,3-D may be because it is more effective in preventing nematode attack of the roots, which slows plant development, but could also be due to its effect of increasing nitrogen mineralisation and so promoting increased plant growth. Grove *et al.* (1999*a*) noted that

root invasion of young potato plants can led to a very early reduction in plant nutrient uptake with an associated reduction in plant growth.

4.4.2 Percentage ground cover

Santé had a significantly higher percentage ground cover than Estima on all of the dates when an assessment was made which would be expected since its emergence was more advanced. The percentage ground cover varied greatly between treatments and the stunting was so severe for some of the treatments that the canopy did not merge and form 100% ground cover. The ground cover of plants at 54 days after planting was 50.1% compared with only 22.1% for those untreated with 1,3-D. The high population density of nematodes in the untreated plots caused severe stunting of the plants due to large amounts of damage caused after invasion. This decreased growth was also seen in the plant growth analysis. Trudgill *et al.* (1975*a,b*) noted that PCN slows the rate of leaf expansion and so decreases the efficiency of light interception. Since the total amount of light intercepted by photosynthetic leaves is one of the determining factors for yield, then this will lead to a reduction in yield (Trudgill *et al.* 1998). Decreased top growth in the early part of the season and premature senescence are thought to account for much of the reduction in yield due to PCN (Trudgill, 1986; Haverkort & Trudgill, 1995),

4.4.3 Root invasions

The root invasion of plants was much higher than in the previous experiment but this would be expected since it also had a higher initial population density. The lowest root invasion was for Santé treated with 1,3-D and full-rate oxamyl while the highest was for Estima with only half-rate oxamyl. It was observed that 1,3-D decreased root invasion and as would be expected since the fumigation would have been able to kill many of the

nematodes within the cyst. It is surprising that oxamyl had no effect on invasion per g root. However, oxamyl did reduce invasion when it was measured per plant rather than per g root. The calculation of invasion per plant was done so that a comparison could be made properly between cultivars since Santé had a larger rooting system than Estima (Table 4.19). The results still showed that Santé had lower invasion than Estima. The mechanisms of plant resistance and the invasion of resistant plants have been studied by other workers. Little is known about how the different sources of PCN resistance act to prevent nematode increase, (Finlay *et al.*, 1998). However plant resistance does not usually decrease rates of hatch and invasion (Trudgill *et al.* 1998). Turner & Stone (1984) found a decrease in the rate of nematode development occurred with resistance and Phillips *et al.*, (1982) found differences in the number of juveniles within the roots of a more susceptible compared to a highly resistant genotype. Other reports indicate that juveniles may leave the roots of resistant plants. Forrest, Trudgill & Cotes (1986) found that although the invasion rates were similar for both susceptible and resistant potato cultivars, more second stage juveniles re-emerged from the roots of the resistant cultivars. This has also been suggested by Forrest *et al.* (1984) and Mullin & Brodie (1988*a*). Therefore, in the work reported here, it is possible that juveniles which had invaded Santé had subsequently left the root system because of its resistance.

4.4.4 Growth analysis

Total plant weight was greater for Santé than Estima since it had significantly greater stem, root and stolon weights and the top weight was non-significantly greater. There was an interaction between cultivar and 1,3-D, where 1,3-D significantly increased the total plant weight of Santé more than Estima -largely due to increases in the top weight. Total plant

weight was significantly increased by 1,3-D and by oxamyl, largely due to increases in the top weight but also in the stolon weight.

Studies in the past have demonstrated that PCN damage decreases top growth of infested plants (Trudgill & Cotes, 1983*a*). The effects on top growth were also quantified by measuring the percentage ground cover. This measurement is often more meaningful in relation to tuber yields than top weight measurements, because it is directly correlated with light interception (Trudgill *et al.*, 1998). Top weight and percentage ground cover are more closely correlated when the plants are smaller.

At 44 days after planting the top weight was significantly increased by 1,3-D, as was the ground cover at 47 days after planting. There was a good correlation between yield and ground cover (Fig. 3.4). Oxamyl linearly increased top weight and ground cover. There was no significant difference in top weight between cultivars although Estima had significantly greater ground cover. There were interactions between cultivars and 1,3-D for both ground cover and top weight with the response being greater for Estima in both cases. The results from the top growth assessments and the percentage growth cover assessments confirm that 1,3-D and oxamyl increased the vigour of plants in treated plots.

The number of tubers set by plants is determined by stem density, spatial arrangement, variety and environment (Allen & Wurr, 1992) and therefore make important contributions to final yield. Harris (1992) concluded that a plant density scale based on the number of stems relates to tuber yields better than any alternative. It is therefore worth noting that 1,3-D increased both the number and weight of stems for both cultivars. The weight of stems would be expected to be greater since the top weight and percentage ground cover

were greater. It is more unexpected that the numbers of stems were increased, but this could be due to the effect of nitrogen mineralisation by 1,3-D making the plant more vigorous. Interestingly, oxamyl linearly decreased the weight and number of stems of Estima but increased the number and weight of stems of Santé. Why oxamyl should have the opposite effect on each cultivar is unclear. One explanation is that the number of main stems per plant were assessed and it is possible that oxamyl caused the Estima plants to produce fewer main stems and more secondary stems per plant while having the opposite effect on Santé.

Previous work has found that plants heavily infested by PCN have a less extensive root system. The root weight and the total root length of plants are both decreased (Evans *et al.*, 1977). 1,3-D had no effect on root weight while the plants untreated by oxamyl had a higher root weight than those treated. This is not what would be expected and may be explained by the reduction in root length caused by nematode attack. In plants that are protected from attack by oxamyl, the roots may have been longer and therefore penetrated deeper down the soil profile. It is possible that when the plants were being harvested for analysis, that some of the deeper roots were lost. This would explain why the root weight decreased. Treatment with 1,3-D reduced root invasion so would also have been expected to increase the root weight but, since this was not observed, the same reason may apply.

Previous studies have shown that PCN decrease top growth proportionately more than root growth, with top/root ratios being decreased up to threefold (Trudgill & Cotes, 1983*a*). 1,3-D increased the ratio twofold indicating that, although the root weight was unchanged by treatment, the overall plant weight was increased by an increase in the top weight.

Oxamyl increased the ratio as would be expected since treated plots had lower root weights and higher top weights.

4.4.5 Incidence of *R. solani*

The assessments for *R. solani* were made on all plots and the means were calculated from values derived from two cultivars, two levels of 1,3-D and three levels of oxamyl. All three of these factors may have affected stem canker incidence, although no statistically significant differences were observed. The analysis did show evidence for an interaction between cultivar and oxamyl with oxamyl decreasing stem canker for Estima but increasing it for Santé, although the effect was not statistically significant. The growth analysis results from the second experiment showed that the number of stems was similar for both cultivars but that the weight of stems was significantly less for Estima. There were significant linear interactions between cultivar and oxamyl with the number and weight of stems decreased by the use of oxamyl for Estima but increased for Santé. However, it seems unlikely that the effects on the number and weight of stems seen as a result of the use of oxamyl can be attributed to the control of *R. solani* as the effects were opposite to what would be expected.

The results from the growth analysis showed that the use of 1,3-D significantly increased the number and weight of stems. It is possible that this increase in weight was due in part to a decrease in stem canker. However, estimations of correlation's between the incidence of stem canker and the number and weight of stems were not significant.

Since the infection of shoots by *R. solani* soon after planting delays stem emergence (Harris, 1992) and development of foliage, incidence of stem canker was used as a

covariate in the analysis of variance done for emergence and percentage ground cover assessments. The covariate was found not to be significant for any of the assessments made on either emergence or percentage ground cover. In addition, incidence of stem canker was used as a covariate in the analysis of variance of yield but again it was found not to be significant.

Hide *at al.* (1985*a*) found that sprouting seed tubers hastened shoot emergence and slightly decreased stem canker. Hide & Firmager (1989) suggested that delayed emergence which prolongs the time that potato shoots are in the soil and susceptible to infection are likely to increase stem canker. Therefore the effect of 1,3-D in advancing emergence may be another mechanism by which it can reduce stem canker.

Previous work by Altman & Fitzgerald, (1960) had found that 1,3-D had reduced *Rhizoctonia* infection. However, Sumner *et al.* (1997) found that 1,3-D was ineffective at controlling a range of soilborne pathogenic fungi including *R. solani* and Read & Hide (1995) found that 1,3-D increased the prevalence of *R. solani* on tuber eye tissue plugs. Thus, although the results from both experiments reported here show a trend for the reduction in stem canker on potatoes following treatment with 1,3-D, further experiments are necessary before any conclusions can be made.

4.4.6 Yield of tubers

It was expected that Santé, with its full resistance to *G. rostochiensis* and partial resistance to *G. pallida,* would have higher yields than Estima. The range in the total yields was over six-fold for the twelve individual treatments. The lowest yields were for untreated Estima plots, which produced only 8.6 t ha^{-1}, while the highest yields were in Santé plots treated

with 13-D and oxamyl (57.2 t ha^{-1}). The population density of 190 eggs g^{-1} soil essentially caused crop failure in the untreated Estima plots. In contrast, the Santé plots untreated with 1,3-D and oxamyl yielded relatively well. In addition, the percentage ware yield was 93.0% for Santé compared with only 64.8% for Estima. An aim of management tactics for PCN is to prevent a reduction in yield loss. Overall yields are a useful measure but for strategic management, the use of ware grade yields is more useful in terms of assessing how successful an approach has been. The production of tubers of non-ware grade is a waste of assimilates, which could have gone into producing fewer, larger tubers.

The results from the untreated controls show that the total yield for Estima in the control plots was only 8.6 t ha^{-1} but was 33.4 t ha^{-1} for Santé (Appendix 12). These results are similar to those reported by Evans (1982) who found that the yield of resistant and non-resistant cultivars varied greatly when grown on site infested with *G. rostochiensis*. The initial population density of 105 eggs g^{-1} soil was similar to that here. Pentland Dell yielded only 13.4 t ha^{-1} and the resistant cultivar Cara 54.6 t ha^{-1}. At an initial population density of only 8 eggs g^{-1} soil Pentland Dell yielded 46.3 t ha^{-1} and Cara 59.4 t ha^{-1}. These results show that in heavily infested plots, the yield of Cara was reduced by 4.8 t ha^{-1} compared with 32.9 t ha^{-1} for Pentland Dell. The NIAB recommended variety list (Anon, 2000) rates Estima and Santé as equal for yield and so the massive differences in yields in the untreated plots between the cultivars shows that Estima in this experiment had a much lower tolerance to PCN attack than Santé.

The application of 1,3-D significantly increased ware yields, total yields and percentage ware yields. The increase in total yield from using 1,3-D was nearly twofold (22.8 t ha^{-1}) as was the increase in ware yield (21.1 t ha^{-1}). 1,3-D increased the percentage of tubers of

ware grade from 72.6% to 86.3% and this more favourable size distribution would increase the value of the crop. This was also found by Barker *et al.* (1998). Large yield increases as a result of the application of 1,3-D were also found by Whitehead & Nichols (1992*a*) and Whitehead *et al.* (1994).

It had been found that the increase in yield as a result of the application of 1,3-D is at least partly due to its effects on nitrogen mineralisation since fumigants such as 1,3-D can effect soil microorganisms involved with soil fertility (Martin & Pratt, 1958). Many studies have shown that soil fumigants increase nitrogen mineralisation (Wolcott *et al.*, 1967; Jenkinson & Powlson, 1970; Williams & Salt, 1970; Rovira, 1976; Elliot *et al.*, 1974; Tu, 1996).

The increase in yield as a result of the application of 1,3-D would also be due to its effect in reducing the initial population densities of PCN which would lead to reduced root invasion. The results for root invasion show that 1,3-D reduced root invasion from 4037 juveniles g^{-1} root to 1192 juveniles g^{-1} root. Damage caused by the invasion of PCN to roots slows root extension and decreases the efficiency of root function, resulting in chronic deficiencies of the least available nutrients (Trudgill *et al.* 1998). This slows the rate of haulm expansion and decreases the efficiency of light interception of the plant leading to a decrease in yield (Trudgill *et al.* 1998). Therefore, the effect of 1,3-D in increasing yields is due partly to its effect in controlling PCN but also due to the additional benefit of increased nitrogen mineralisation.

Brown (1983) predicted a loss of 6.2 t ha^{-1} for each 20 eggs g^{-1} soil as an average for a range of cultivars. The results from this first field experiment show a yield of 28.6 t ha^{-1} for the control plots of Estima at an initial population density of 100 eggs g^{-1} soil. In this

second field experiment, the yield for the untreated Estima at an initial population density of 190 eggs g^{-1} soil was only 8.6 t ha^{-1}. This is a reduction of 4.4 t ha^{-1} for every increase of 20 eggs g^{-1} soil compared to the first experiment and is clearly less than was predicted by Brown (1983). However, the figures predicted by Brown (1983), were average figures and at such high population densities, it would be expected that there would be a range of differences. These could be due to effects of the different soil types. Trudgill (1986) found that there is considerable variation between sites after reviewing the relationship between yield losses caused by PCN and the increase in yields resulting from nematicide treatment.

4.4.7 Number of tubers at harvest

The total number of tubers was similar for both cultivars although the number of ware grade tubers was significantly greater for Santé. 75.4% of tubers of Santé were of ware grade compared to only 40.6% for Estima. This, combined with the yield results, shows that Estima produced a greater number and yield of non-ware grade tubers.

The total number of tubers, the number of tubers of ware grade and the percentage of tubers of ware grade were all significantly increased by the application of 1,3-D (Tables 4.30, 4.34, 4.36). This is similar to the results found in the first field experiment but is again in contrast to Barker *et al.* (1998) who found that 1,3-D reduced tuber numbers. The results also show that the mean tuber weight was increased by 1,3-D so the effect on tuber numbers was not at the expense of the size of tubers. Tuber size distribution was favourably shifted to increase the number of tubers in ware grades. This was also found by Barker *et al.* (1998). However, the number of tubers of non-ware grade was unaffected by 1,3-D. Oxamyl linearly increased the number of tubers but didn't increase the percentage that were of ware grade.

4.4.8 Nematode population densities

The overall Pi of 190 eggs g^{-1} soil was an extremely high initial population density and would have required use of a both fumigation and a granular nematicide to avoid yield loss. This level of infestation and its uniformity made the site suitable for this experiment. The Pf values were only significantly affected by Santé, which caused massive reductions in numbers of nematodes. Santé gave an overall Pf of 11 eggs g^{-1} soil compared to 207 eggs g^{-1} soil for Estima. This is similar to work by Grove *et al.* (1999*b*) who found that Santé reduced a population containing predominantly *G. rostochiensis* but some *G. pallida* from 76 eggs g^{-1} soil to 7 eggs g-1 soil. Grove *et al.* (1999*b*) also found that Santé reduced the mean population densities from 90 eggs g^{-1} soil to 8 eggs g-1 soil on a mixed site containing *G. pallida* and *G. rostochiensis*.

For the Pf values and the Pf/Pi ratios, there was a significant interaction between cultivar and 1,3-D, with Santé having lower values when used with 1,3-D but Estima having increased values. The use of 1,3-D would be expected to reduce the Pf and Pf/Pi values and this increase for Estima is possibly explained by the fact that Estima suffered almost crop failure in plots untreated by 1,3-D. It is possible that 1,3-D increased the vigour of the plants and thus increased the multiplication rates of the nematodes. Estima that were in plots treated with 1,3-D suffered much less root invasion and were healthier plants that were able to produce better feeding sites for the nematodes and so this could lead to greater multiplication.

Although the use of 1,3-D and oxamyl had no significant effect on Pf/Pi ratios individually, there was a significant linear interaction between the use of 1,3-D and oxamyl

for the Pf/Pi ratios. It was found that the combined use of 1,3-D and full-rate oxamyl gave significantly lower PF/Pi ratios than treatment with 1,3-D alone or with full-rate oxamyl alone. This interaction clearly shows a benefit for the combined use of fumigant and granular nematicides.

4.4.9 Economic benefits from nematicide use

An economic analysis of the benefits of the use of the different treatments was again undertaken. Values for the variable costs and price of potatoes was obtained from the Farm Management Pocketbook (Nix, 2000) where the five-year average price for maincrop tubers was given as £131 per tonne. The calculations also assume the same value of the crops produced for each of the two cultivars.

The calculations shown in Table 4.40 reveal that for Estima, all treatments without 1,3-D had negative gross margins. The least profitable was the untreated control which showed a loss of £1476. The use of 1,3-D greatly increased the gross margins for all levels of oxamyl and the highest gross margin was for 1,3-D with full-rate oxamyl (£1484).

For Santé, in both the presence and absence of 1,3-D, the untreated control was more profitable than the use of full-rate oxamyl. The use of 1,3-D greatly increased the gross margins when used for all levels of oxamyl and gave an increase of £1884 for the control and an increase of £2002 for full-rate oxamyl.

The results for both cultivars show that there is a clear economic benefit from the use of 1,3-D. For Estima, in the presence of full-rate oxamyl, the use of 1,3-D increased the gross margin by £2644 compared with £2002 for Santé.

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Table 4.40. The gross margins for each of the twelve treatments

Cultivar	1,3-D[a]	Oxamyl rate[b]	Variable costs (£ ha^{-1})	Fumigant cost (£ ha^{-1})	Granules cost (£ ha^{-1})	Total variable cost (£ ha^{-1})	Ware (t h
Estima	-	zero	2000	0	0	2000	
	-	half	2000	0	176	2176	8
	-	full	2000	0	352	2352	9
	+	zero	2000	579	0	2579	2
	+	half	2000	579	176	2755	2
	+	full	2000	579	352	2931	3
Santé	-	zero	2000	0	0	2000	3
	-	half	2000	0	176	2176	3
	-	full	2000	0	352	2352	3
	+	zero	2000	579	0	2579	5
	+	half	2000	579	176	2755	4
	+	full	2000	579	352	2931	5

[a, b] see Table 4.1 for actual rates of product used

The use of oxamyl was of economic value to Estima but not to Santé. The use of full-rate oxamyl was of more benefit than half-rate to Estima. The NIAB recommended variety list (Anon, 2000) rate both Estima and Santé as having similar yield potentials. It is therefore likely that some of the observed yield differences are due to differences in their tolerance to high levels of PCN. It is observed that the yield of untreated control of Santé had a larger gross margin than the use of Estima with both 1,3-D and full-rate oxamyl.

It is also worth noting that although the five-year average price for maincrop potatoes was £117 per ton (£141 in 2001 money values), the average price varied from £66 to £157. Similar calculations to those in Table 4.40 were done based on the value of the crop at £66 to £157 per ton. If the value of the crop was only £66, then all treatments for Estima had negative gross margins. For Santé, the use of 1,3-D with all levels of oxamyl produced gross margins ranging from £534 to £728. However, if the value of the crop was taken as £157, the use of 1,3-D when used with oxamyl would produce a gross margin of £2360 for Estima and £5312 for Santé.

4.4.10 Conclusions

The first objective was to determine whether a resistant cultivar was more effective than a combination of 1,3-D and a granular nematicide for the control of PCN multiplication and for increasing yield. The Pf/Pi ratios show that the resistant cultivar significantly reduced nematode multiplication compared to the susceptible cultivar whereas the use of 1,3-D and oxamyl did not significantly reduce the Pf/Pi ratios. However, there was a significant linear interaction between the use of 1,3-D and oxamyl and it was found that the combined use of 1,3-D and full-rate oxamyl gave significantly lower PF/Pi ratios than treatment with 1,3-D alone or with full-rate oxamyl alone. The ware yield of untreated Estima (4.0 t ha^{-1}) was

greatly increased by the combined use of 1,3-D and oxamyl (33.7 t ha^{-1}). and the yield for untreated Santé (31.3 t ha^{-1}) was also greatly improved by the combined use of 1,3-D and oxamyl (52.5 t ha^{-1}). Therefore, the results show that the resistant cultivar Santé grown without nematicides yielded as much as the susceptible cultivar Estima grown on plots treated with both fumigant and granular nematicides. However, in both cases, yields were about 20 t/ha less than that of cultivar Santé on treated plots.

The second objective was to determine whether the use of full-rate oxamyl was more effective than half-rate oxamyl for control of PCN multiplication and for increasing yield. There were no significant differences in Pf/Pi ratios between full or half-rate oxamyl and neither of the oxamyl treatments significantly decreased the Pf/Pi ratio compared to the control. Although the ware yields of full-rate oxamyl was higher than that of half-rate oxamyl, the difference was not statistically significant.

The final objective was to determine whether the combined use of a resistant cultivar, 1,3-D and a granular nematicide was the most effective strategy for the control of PCN multiplication and for increasing yield. The Pf/Pi results show that the resistant cultivar was the most effective method for control of nematode populations and the yield results show that even with the use of a resistant cultivar, significant yield increases were obtained from fumigation. The use of oxamyl had no significant effect on multiplication or yield when used with Santé. However, the experimental site contained mainly *G. rostochiensis,* which is fully controlled by Santé. In situations where *G. pallida* is present, it would be expected that a granular nematicide would have a greater effect on yield and nematode multiplication.

5.0 Chapter 5.

General discussion

5.1 General discussion and conclusions

5.1.1 The occurrence and distribution of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* in England and Wales

The main aims of this part of the research were to estimate the proportion of land which is used to grow potatoes that is infested with PCN and to determine the relative abundance of the two species. Once a range of PCN populations had been collected, the secondary aim was to compare results for the detection, quantification and identification of PCN populations using standard counting, PCR, IEF, ELISA and bait plant techniques.

The results show that *Globodera* cysts were found in 64% of samples tested. Since this is significantly higher than the precious estimates (Hancock, 1996), the results suggest that the PCN problem in England and Wales is therefore increasing. The decrease in the number of potato growers has meant that production has become more specialised and has been concentrated on a smaller area of land leading to shorter rotations (Anon, 1997; Haydock & Evans, 1998). The results of the survey found that just over 50% of the sites sampled had a rotation length of 1 in 5 or lower. The shortening of rotations will reduce population decline for many growers. In the absence of host crops, *G. pallida* may take as long as 18 years to decline to a non-damaging level compared with 10 years for *G. rostochiensis* (Evans, 1993).

The standard cyst counts at Harper Adams revealed that 56% of the field samples tested contained PCN, but the second standard cyst count and bait plant tests at Rothamsted increased the detection by 8% to 64%. These results strongly suggest that many PCN infestations are cryptic and may not be detected by conventional sampling and extraction methods.

The population densities of the infestations found were similar to those obtained by Hancock (1988) and mainly low with about 62% below 10 eggs g^{-1} soil and only 6% above 60 eggs g^{-1} soil. The results indicate that most growers do not have significantly high populations.

The species identification results revealed that 8% of populations were *G. rostochiensis*, 67% were *G. pallida* and 25% contained both species. These results are similar to those found by ADAS (Hancock, 1996) and clearly show that *G. pallida* is the predominant species in England and Wales. Since earlier surveys estimated a lower incidence for *G. pallida* (Dixon *et al.*, 1968; Brown, 1970), this suggests that growing cultivars resistant to *G. rostochiensis* has selected *G. pallida* in field populations. Cultivars with partial resistance to *G. pallida* represented only 6% of the total number of plantings while those with resistance to *G. rostochiensis* represented 43% from the survey sites. This is similar to plantings by Potato Marketing Board registered producers (Anon, 1997) and reflects market demands for choice of varieties.

The secondary aim of this study was to compare IEF, ELISA and PCR-based techniques for species determination of field populations of PCN. All methods were able to distinguish the two species of PCN and all were also able to register population mixtures. PCR gave a greater number of positive results. ELISA failed to agree with other methods on the identification of pure and mixed samples of *G. pallida*. The disagreement between the results obtained in this study may be explained by differences in the principles of operation and the sensitivity of each of the methods. The sensitivity of PCR is much greater than isoelectric focusing which requires large numbers of viable eggs. Low

viability cysts often contain insufficient protein for diagnosis (Fleming *et al.*, 1998). Both sets of PCR primers failed to yield amplification products from some field samples known to contain eggs. The same set of primers (Mulholland *et al.*, 1996) also failed previously to detect PCN DNA from field samples known to contain live eggs (Evans *et al.*, 1998). Several factors may affect the amplification of DNA in PCR. These include the inhibition of DNA amplification at high concentrations of template DNA, heterogeneity in the ITS sites (Bulman & Marshall, 1997), genetic differences within the species G. *pallida* (Fleming & Marks, 1983), genomic variation (Burrows *et al.*, 1996; Phillips *et al.*, 1998) or the presence of different pathotypes in the same sample (Kort *et al.*, 1977; Stone *et al.*, 1986).

All of the methods were laborious but all were much faster than a bait plant test. However, PCR and IEF results can be obtained in one day, whereas ELISA results are only obtained in two days. The costs of reagents for a PCR reaction (£0.35 per sample) and that of an ELISA (£0.20 + MAbs) is similar too. The sensitivity and specificity of the PCR and ELISA make them obvious choices for determination and quantification of PCN species in field populations.

5.1.2 The use of 1,3-dichloropropene in combination with granular nematicides for the control of potato cyst nematodes

The aims of the experiment were to compare the use of 1,3-D in combination with the granular nematicides aldicarb, oxamyl and fosthiazate for the control of PCN and to increase yields. The results for plant emergence showed that, although emergence was advanced by 1,3-D, aldicarb and oxamyl, the final emergence was unaffected by any of the treatments. The ground cover was significantly increased by 1,3-D and by all three

granular nematicides, which suggests that the nematicides were able to reduce the damage caused by PCN. This is supported by work by Grove *et al.* (1999*a,b*) who found that plant emergence was advanced by the use of granular nematicides. This earlier emergence led to increased ground cover and it was found that the effect of treatment on ground cover increased over time. Regression analysis of yield against ground cover showed an increase in yield with an increase in ground cover. This agrees with Trudgill (1986) who commented that decreased top growth in the early part of the season and the premature senescence in the late part of the season account for much of the reduction in yield.

The invasion of plant roots by juveniles was significantly decreased by the autumn application of 1,3-D and by all three granular nematicides. There was a highly significant correlation between root invasion and yield and it appears that the reduction in damage caused by root invasion will account for the differences in yield that were observed. This is supported by Evans *et al.* (1977) and Trudgill *et al.* (1975*a,b*) found that root invasion per g root is related to crop yield and as invasion increases, the yield decreases in the majority of cases.

The application of 1,3-D and the use of the granular nematicides significantly increased yields with a large range between treatments (28.6 – 51.4 t ha^{-1}). This has previously been found by Whitehead & Nichols (1992*a*) and Whitehead *et al.* (1994). The percentage of tubers of ware grade was increased by all of the spring treatments, indicating that there had been a favourable shift in the tuber size distribution. The total number of tubers and the percentage of tubers of ware grade were all significantly increased by the autumn application of 1,3-D and the tuber size distribution was again favourably affected and this had previously been reported by Barker *et al.* (1998).

It has been shown that soil fumigants retard or inhibit nitrification of ammonium nitrogen (Wolcott, Liao & Kirkwood, 1967; Jenkinson & Powlson, 1970). Williams & Salt (1970) found that mineralised nitrogen increased after treatment with D-D (1,2-dichloropropane, 1,3-dichloropropene) and Tu (1996) found that Telone C (1,3-dichloropropene and chloropicrin) decreased nitrification activity. Therefore, the increase in yield as a result of the use of 1,3-D is partly due to its effects on nitrogen mineralisation.

There was a nearly ten-fold difference in the range in Pf values, depending on treatment. The lowest Pf value was from treatment with 1,3-D and aldicarb and the highest was from the spring application of 1,3-D. The lowest Pf/Pi ratios were for the combination of 1,3-D with oxamyl and aldicarb, while the highest was for the spring application of 1,3-D. The use of 1,3-D when used in combination with a spring treatment significantly reduced nematode multiplication. This agrees with Whitehead *et al.* (1994) who found that nematode multiplication was controlled better in plots treated with 1,3-D and a granular nematicide (ethoprophos) than in plots treated with ethoprophos alone. The combination of 1,3-D with oxamyl and aldicarb had the lowest invasion and Pf/Pi ratios and the highest yields which suggests that the prevention of nematode invasion and multiplication reduces plant damage and leads to increased yields.

5.1.3 The use of 1,3-dichloropropene in combination with the resistant cultivar Santé and oxamyl at full and half-rates for the control of potato cyst nematodes

The aims of the experiment were to assess the various treatment combinations for the control of nematode multiplication and to increase yields. The results show that an integrated approach to nematode control on heavily infested sites can lead to significant

decreases in nematode population levels and give economic yield benefits. 1,3-D increased the final emergence and advanced the emergence for both cultivars but more for Santé than Estima. Oxamyl advanced the emergence but the effect was shorter-lived than the effect of 1,3-D. The greater effect of 1,3-D may be because it is more effective in preventing nematode attack of the roots. The advancement in emergence between cultivars and treatment was also seen in the ground cover assessments. Trudgill (1986) commented that these differences early in the season and early senescence later in the season account for much of the reduction in yield due to PCN.

The high population density of nematodes caused the root invasion of plants to be high. The lowest root invasion was for Santé with 1,3-D and full-rate oxamyl while the highest was for Estima with only half-rate oxamyl. 1,3-D decreased root invasion as did oxamyl when it was measured per plant rather than per g root showing that both chemicals were effective for the control of nematodes. The high population density of nematodes caused severe stunting of the plants in the untreated plots and consequently reduced the percentage ground cover.

The growth analysis showed that the application of 1,3-D and oxamyl both increased total plant weight and top weight. 1,3-D increased stolon weight, number of stems and weight of stems. This advantage was seen in the yield results where 1,3-D significantly increased ware yields, total yields and percentage ware yields. The increase in total yield from using 1,3-D was nearly twofold as was the increase in ware yield. 1,3-D also gave a more favourable size distribution as reported by Barker *et al.* (1998). The total number of tubers, the number of tubers of ware grade and the percentage of tubers of ware grade were all significantly increased by the application of 1,3-D.

The Pf values were only significantly affected by Santé, which caused massive reductions in numbers of nematodes. This has also been reported by Grove *et al.* (1999*a,b*). The use of 1,3-D would be expected to reduce the Pf and Pf/Pi. It is possible that 1,3-D increased the vigour of the plants and thus increased the multiplication rates of the nematodes. The results reported here are supported by Phillips & Trudgill (1998) who commented that a combination of a resistant cultivar and a nematicide is needed to bring higher PCN levels under control and that nematicides are more effective at preventing further increase in small populations of PCN than they are at decreasing large ones.

5.1.4 The effect of the use of the soil fumigant 1,3-dichloropropene on the germination and growth of weed seeds

The aim of this work was to assess the effectiveness of 1,3-D for the control of weeds at rates used for PCN control. There was a wide range of weed species on the experimental site but mostly annual broad-leaved weeds. The numbers of weeds that had germinated and were growing were greatly reduced in soil after fumigation with 1,3-D and the percentage of ground covered by weeds was decreased by the use of 1,3-D. Overall, the results show that 1,3-D has an effect on germinating weeds in soil after fumigation, with some six-times fewer germinating after treatment. The results for the germination tests only showed a two-fold decrease in the number of viable seeds germinating. It would therefore appear that the main effect of 1,3-D is a phytotoxic effect on young seedlings in treated soil. The effect on the weed seed bank is less strong since seeds are harder to kill than growing plant tissues. This work would also agree with other workers who have reported the control of weeds by 1,3-D (Altman & Fitzgerald, 1960; Turner *et al.*, 1974; Coupland & Peabody, 1980; Bond & White, 1984).

5.1.5 The effect of the use of the soil fumigant 1,3-dichloropropene on the incidence of *Rhizoctonia solani* Kühn on potatoes

The incidence of diseased stems of *R. solani* was reduced by the application of 1,3-D although the differences were not statistically significant. Both cultivar and oxamyl may have affected stem canker incidence, although no statistically significant differences were observed for either. The results from the growth analysis in the second experiment showed that the use of 1,3-D significantly increased the number and weight of stems. It is possible that this increase in weight was due in part to a decrease in stem canker. However, estimations of correlations between the incidence of stem canker and the number and weight of stems were not significant. Hide *at al.* (1985*a*) found that a relationship between shoot emergence and a decrease in stem canker and it was suggested by Hide & Firmager (1989) that delayed emergence which prolongs the time that potato shoots are in the soil and susceptible to infection are likely to increase stem canker. Therefore 1,3-D may reduce stem canker by advancing emergence. The results from both experiments show a trend for the reduction in stem canker on potatoes following treatment with 1,3-D. However, further experiments would be necessary to find differences which are statistically different.

5.2 Further Research

5.2.1 The occurrence and distribution of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* in England and Wales

The results from the survey showed the occurrence and distribution of PCN in England and Wales. However, assembling PCN data from commercial organisations that hold historical data on species determinations and population densities could expand the information currently held. This data could be used to create a database and to create maps of species

distribution at the parish level. Comparisons of average population levels in different areas could be made in addition to simple presence or absence mapping.

An other area of further study is to investigate the difference in results obtained between the different sets of primers for the PCR reactions. PCR was shown to have greater sensitivity than conventional species determination using IEF but the use of PCR as a standard diagnostic tool would require these differences to be resolved. Further work could also be done on the ELISA-based system make it more specific for each species of PCN.

5.2.2 The use of the soil fumigant 1,3-D for the control of potato cyst nematodes

The research showed that the spring application of 1,3-D was effective in the second field experiment but not in the first. Further work could be done to look at spring application of 1,3-D when compared to the autumn application of 1,3-D. In particular, an experiment could be done to assess the effect of timing of fumigant application on soil nitrification and the subsequent effect on yield.

The high cost of fumigant nematicides such as 1,3-D mean that they are not as widely used as granular nematicides. The combined use of fumigant and granular nematicides require very high population levels that would cause severe yield losses before they can be economically justified. Therefore, an area of further research could be the field-scale testing of spatial applications of fumigant nematicides. The effects of the treatments would be monitored. The aim of the research would be to test and develop different soil sampling strategies which would be accurate enough so patch applications of fumigant nematicides could be made.

5.2.3 The effect of the use of the soil fumigant 1,3-dichloropropene on the germination and growth of weed seeds

The work already done has demonstrated that 1,3-D can have an effect on the weed seed bank. Further research could be to done on individual weed species. Methods of improving the soil of the surface could be done such as by sealing. The most common weed species in arable land would be studied first.

5.2.4 The effect of the use of the soil fumigant 1,3-dichloropropene on the incidence of *Rhizoctonia solani* Kühn on potatoes

The assessments that were made showed that there was a trend for the decrease in levels of *R. solani* by the use of 1,3-D. Further experiments designed specifically to investigate the relationship between *R. solani* by the use of 1,3-D would be necessary. A larger experiment with a higher number of assessments made on each plot would be necessary.

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Appendices

APPENDICES NOT DIGITISED AT THE REQUEST OF THE AWARDING UNIVERSITY